



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/82, A01H 5/00 // C12N 9/10	A1	(11) International Publication Number: WO 98/35051 (43) International Publication Date: 13 August 1998 (13.08.98)
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(21) International Application Number: PCT/CA98/00055

(22) International Filing Date: 5 February 1998 (05.02.98)

(30) Priority Data:

60/036,946	10 February 1997 (10.02.97)	US
08/868,786	4 June 1997 (04.06.97)	US

(71) Applicant (for all designated States except US): HER MAJESTY THE QUEEN IN RIGHT OF CANADA as represented by THE DEPARTMENT OF AGRICULTURE AND AGRI-FOOD CANADA [CA/CA]; P.O. Box 3000, Main, Lethbridge, Alberta T1J 4B1 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KAWCHUK, Lawrence, Michael [CA/CA]; 905 - 22 Avenue, Coaldale, Alberta T1M 1B5 (CA). ARMSTRONG, John, David [CA/CA]; 2422 - 12 Avenue South, Lethbridge, Alberta T1K 0P5 (CA). LYNCH, Dermot, Roborg [CA/CA]; 3821 Glacier Avenue South, Lethbridge, Alberta T1K 3N9 (CA). KNOWLES, Norman, Richard [CA/CA]; 3507 Hillview Crescent, Edmonton, Alberta T6L 1P4 (CA).

(74) Agent: McKAY-CAREY, Mary, Jane; McKay-Carey & Company, 2125 Commerce Place, 10155 - 102nd Street, Edmonton, Alberta T5J 4G8 (CA).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

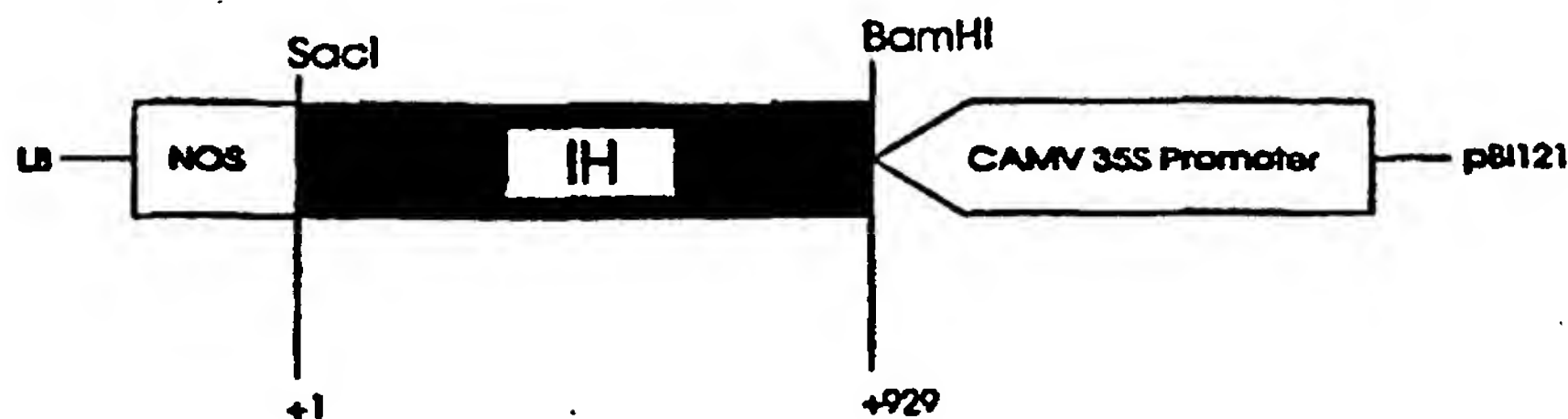
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: TRANSGENIC POTATOES HAVING REDUCED LEVELS OF ALPHA GLUCAN L- OR H-TYPE TUBER PHOSPHORYLASE ACTIVITY WITH REDUCED COLD-SWEETENING

(57) Abstract

Potato plants which exhibit reduced levels of α glucan L-type tuber phosphorylase (GLTP) or α glucan H-type tuber phosphorylase (GHTP) enzyme activity within the potato tuber are provided. The conversion of starches to sugars in potato tubers, particularly when stored at temperatures below 7 °C, is reduced in tubers exhibiting reduced GLTP or GHTP enzyme activity. Reducing cold-sweetening in potatoes allows for potato storage at cooler temperatures, resulting in prolonged dormancy, reduced incidence of disease, and increased storage life. Methods for producing potato plants which produce tubers exhibiting reduced GLTP or GHTP enzyme activity are also provided. Reduction of GLTP or GHTP activity within the potato tuber may be accomplished by such techniques as suppression of gene expression using homologous antisense RNA, the use of co-suppression, regulatory silencing sequences, chemical and protein inhibitors, or the use of site-directed mutagenesis or the isolation of alternative alleles to obtain GLTP or GHTP variants with reduced starch affinity or activity.



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5 TRANSGENIC POTATOES HAVING REDUCED LEVELS OF ALPHA GLUCAN L- OR H-TYPE TUBER PHOSPHO-
6 RYLASE ACTIVITY WITH REDUCED COLD-SWEETENING

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This application claims the benefit of U.S. Provisional Patent Application No.
60/036,946 , filed February 10, 1997, which is incorporated in its entirety by reference herein.

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FIELD OF THE INVENTION

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The invention relates to the inhibition of the accumulation of sugars in potatoes by
reducing the level of α glucan L-type tuber phosphorylase or α glucan H-type tuber
phosphorylase enzyme activity in the potato plant.

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BACKGROUND OF THE INVENTION

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Plant stresses caused by a wide variety of factors including disease, environment, and
storage of potato tubers (*Solanum tuberosum*) represent major determinants of tuber quality.
Dormancy periods between harvesting and sprouting are critical to maintaining quality
potatoes. Processing potatoes are usually stored between 7 and 12°C. Cold storage at 2 to
6°C, versus storage at 7 to 12°C, provides the greatest longevity by reducing respiration,
moisture loss, microbial infection, heating costs, and the need for chemical sprout inhibitors
(Burton, 1989). However, low temperatures lead to cold-induced sweetening, and the
resulting high sugar levels contribute to an unacceptable brown or black color in the fried
product (Coffin et al., 1987, Weaver et al., 1978). The sugars that accumulate are
predominantly glucose, fructose, and sucrose. It is primarily the glucose and fructose
(reducing sugars) that react with free amino groups when heated during the various cooking
processes such as frying via the Maillard reaction, resulting in the formation of brown
pigments (Burton, 1989, Shallenberger et al., 1959). Sucrose produces a black colouration
when fried due to caramelization and charring. The ideal reducing sugar content is generally
accepted to be 0.1% of tuber fresh weight with 0.33% as the upper limit and higher levels of

1 reducing sugars are sufficient to cause the formation of brown and black pigments that
2 results in an unacceptable fried product (Davies and Viola, 1992). Although the
3 accumulation of reducing sugars can be slowed in higher temperature (7 to 12°C) storage,
4 this increases microbial infection and the need to use sprout inhibitors. Given the negative
5 environmental and health risks associated with chemical use, development of pathogens
6 resistant to pesticides, and the fact that use of current sprout inhibitors may soon be
7 prohibited, a need exists for potato varieties that can withstand stress and long-term cold
8 storage without the use of chemicals, without the accumulation of reducing sugars, and with
9 greater retention of starch.

10 Carbohydrate metabolism is a complex process in plant cells. Manipulation of a
11 number of different enzymatic processes may potentially affect the accumulation of reducing
12 sugars during cold storage. For example, inhibition of starch breakdown would reduce the
13 buildup of free sugar. Other methods may also serve to enhance the cold storage properties of
14 potatoes through reduction of sugar content, including the resynthesis of starch using reducing
15 sugars, removal of sugars through glycolysis and respiration, or conversion of sugars into
16 other forms that would not participate in the Maillard reaction. However, many of the
17 enzymatic processes are reversible, and the role of most of the enzymes involved in
18 carbohydrate metabolism is poorly understood. The challenge remains to identify an enzyme
19 that will deliver the desired result, achieve function at low temperatures, and still retain the
20 product qualities desired by producers, processors, and consumers.

21 It has been suggested that phosphofructokinase (PFK) has an important role in the
22 cold-induced sweetening process (Kruger and Hammond, 1988, ap Rees et al., 1988, Dixon et
23 al., 1981, Claassen et al., 1991). ap Reese et al. (1988) suggested that cold treatment had a
24 disproportionate effect on different pathways in carbohydrate metabolism in that glycolysis
25 was more severely reduced due to the cold-sensitivity of PFK. The reduction in PFK activity
26 would then lead to an increased availability of hexose-phosphates for sucrose production. It
27 was disclosed in European Patent 0438904 (Burrell et al., July 31, 1991) that increasing PFK
28 activity reduces sugar accumulation during storage by removing hexoses through glycolysis
29 and further metabolism. A PFK enzyme from *E. coli* was expressed in potato tubers and the
30 report claimed to increase PFK activity and to reduce sucrose content in tubers assayed at
31 harvest. However it has been shown that pyrophosphate:fructose 6-phosphate

1 phosphotransferase (PFP) remains active at low temperatures (Claassen et al., 1991). PFP
2 activity can supply fructose 6-phosphate for glycolysis just as PFK can, since the two
3 enzymes catalyse the same reaction. Therefore, the efficacy of this strategy for improving
4 cold storage quality of potato tubers remains in doubt. Furthermore, removal of sugars
5 through glycolysis and further metabolism would not be a preferred method of enhancing
6 storage properties of potato tubers because of the resultant loss of valuable dry matter through
7 respiration.

8 It has also been suggested that ADPglucose pyrophosphorylase (ADPGPP) has an
9 important role in the cold-induced sweetening process. It was disclosed in International
10 Application WO 94/28149 (Barry, *et al.*, filed May 18, 1994) that increasing ADPGPP
11 activity reduces sugar accumulation during storage by re-synthesising starch using reducing
12 sugars. An ADPGPP enzyme from *E. coli* was expressed in potato tubers under the control of
13 a cold-induced promoter and the report claimed to increase ADPGPP activity and lower
14 reducing sugar content in tubers assayed at harvest and after cold temperature storage.
15 However, this strategy does not eliminate starch catabolism but instead increases the rate of
16 starch resynthesis. Thus, catabolism of sugars through glycolysis and respiration occurs and
17 re-incorporation into starch is limited. Up regulation of ADPGPP would not be a preferred
18 method of enhancing storage properties of potato tubers because of the resultant loss of
19 valuable dry matter through respiration. Again, a method involving the reduction of
20 catabolism of starch would be preferable as dry matter would be retained.

21 The degradation of starch is believed to involve several enzymes including α -amylase
22 (endoamylase), β -amylase (exoamylase), amyloglucosidase, and α -glucan phosphorylase
23 (starch phosphorylase). By slowing starch catabolism, accumulation of reducing sugars
24 should be prevented and the removal of sugars through glycolysis and further metabolism
25 would be minimized.

26 Three different isozymes of α glucan phosphorylase have been described. The tuber
27 L-type α 1,4 glucan phosphorylase (EC 2.4.1.1) isozyme (GLTP) (Nakano and Fukui, 1986)
28 has a low affinity for highly branched glucans, such as glycogen, and is localized in
29 amyoplasts. The monomer consists of 916 amino acids and sequence comparisons with
30 phosphorylases from rabbit muscle and *Escherichia coli* revealed a high level of homology,
31 51% and 40% amino acids, respectively. The nucleotide sequence of the GLTP gene and the

1 amino acid sequence of the GLTP enzyme are shown in SEQ ID NO: 1 and SEQ ID NO: 2,
2 respectively. The H-type tuber α -glucan phosphorylase isozyme H (GHTP) (Mori et al.,
3 1991) has a high affinity for glycogen and is localized in the cytoplasm. The gene encodes
4 for 838 amino acids and shows 63% sequence homology with the tuber L-type phosphorylase
5 but lacks the 78-residue insertion and 50-residue amino-terminal extension found in the L-
6 type polypeptide. The nucleotide sequence of the GHTP gene and the amino acid sequence of
7 the GHTP enzyme are shown in SEQ ID NO: 3 and SEQ ID NO: 4, respectively. A third
8 isozyme has been reported (Sonnewald et al., 1995) that consists of 974 amino acids and is
9 highly homologous to the tuber L-type phosphorylase with 81% identity over most of the
10 polypeptide. However, the regions containing the transit peptide and insertion sequence are
11 highly diverse. This isozyme is referred to as the leaf L-type phosphorylase since the mRNA
12 accumulates equally in leaf and tuber, whereas the mRNA of the tuber L-type phosphorylase
13 accumulates strongly in potato tubers and only weakly in leaf tissues. The tuber L-type
14 phosphorylase is mainly present in the tubers and the leaf L-type phosphorylase is more
15 abundant in the leaves (Sonnewald et al., 1995). The nucleotide sequence of the leaf L-type
16 phosphorylase gene and the amino acid sequence of the leaf L-type phosphorylase enzyme are
17 shown in SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

18 The role of the various starch degrading enzymes is not clear, however, and
19 considerable debate has occurred over conflicting results. For example, reduced expression
20 of the leaf L-type phosphorylase (Sonnewald et al., 1995) had no significant influence on
21 starch accumulation. Sonnewald *et al.* (1995) reported that constitutive expression of an
22 antisense RNA specific for the leaf L-type gene resulted in a strong reduction of α glucan
23 phosphorylase L-type activity in leaf tissue, but had no effect in potato tuber tissue. Since the
24 antisense repression of the α glucan phosphorylase activity had no significant influence on
25 starch accumulation in leaves of transgenic potato plants, the authors concluded that starch
26 breakdown was not catalysed by phosphorylases. Considering the high level of sequence
27 homology between identified α glucan phosphorylase isozymes, a similar negative response
28 would be expected with the H-type (GHTP) and L-type tuber (GLTP) isozymes.

29 In view of the foregoing, there remains a need for potato plants which produce tubers
30 exhibiting reduced conversion of starches to sugars during propagation and during storage at
31 ambient and reduced temperatures, particularly at temperatures below 7°C.

SUMMARY OF THE INVENTION

The inventors have found that surprisingly, reduction of the level of α glucan L-type tuber phosphorylase (GLTP) or α glucan H-type tuber phosphorylase (GHTP) enzyme activity within the potato tuber results in a substantial reduction in the accumulation of sugars in the tuber during propagation and storage, relative to wildtype potatoes, particularly at storage temperatures below 10°C, and specifically at 4°C. It is remarkable that, given the complexity of carbohydrate metabolism in the tuber, reduction in the activity of a single enzyme is effective in reducing sugar accumulation in the tuber. The inventors' discovery is even more surprising in light of the previously discussed work of Sonnewald *et al.* (1995) wherein it was reported that reduced expression of the leaf L-type phosphorylase had no significant influence on starch accumulation in leaves of potato plants.

The present invention provides tremendous commercial advantages. Tubers in which cold-induced sweetening is inhibited or reduced may be stored at cooler temperatures without producing high levels of reducing sugars in the tuber which cause unacceptable darkening of fried potato products. Cold storage of tubers storage results in longer storage life, prolonged dormancy by limiting respiration and delaying sprouting, and lower incidence of disease.

Reduction in GLTP or GHTP activity in potato plants and tubers can be accomplished by any of a number of known methods, including, without limitation, antisense inhibition of GLTP or GHTP mRNA, co-suppression, site-directed mutagenesis of wildtype GLTP or GHTP genes, chemical or protein inhibition, or plant breeding programs.

Thus, in broad terms, the invention provides modified potato plants having a reduced level of α glucan L-type tuber phosphorylase (GLTP) or α glucan H-type tuber phosphorylase (GHTP) activity in tubers produced by the plants, relative to that of tubers produced by an unmodified potato plant. In a preferred embodiment, the invention provides a potato plant transformed with an expression cassette having a plant promoter sequence operably linked to a DNA sequence which, when transcribed in the plant, inhibits expression of an endogenous GLTP gene or GHTP gene. As will be discussed in detail hereinafter, the aforementioned DNA sequence may be inserted in the expression cassette in either a sense or antisense orientation. Potato plants of the present invention could have reduced activity levels of either

1 one of GLTP or GHTP independently, or could have reduced activity levels of both GLTP
2 and GHTP.

3 As discussed above, the inventors have found that reduction of activity levels of
4 GLTP or GHTP enzymes in potato plants results in potato tubers in which sugar
5 accumulation, particularly over long storage periods at temperatures below 10°C, is reduced.
6 Therefore, the invention further extends to methods for reducing sugar production in tubers
7 produced by a potato plant comprising reducing the level of activity of GLTP or GHTP in the
8 potato plant. In a preferred embodiment, such methods involve introducing into the potato
9 plant an expression cassette having a plant promoter sequence operably linked to a DNA
10 sequence which, when transcribed in the plant, inhibits expression of an endogenous GLTP
11 gene or GHTP gene. As above, the DNA sequence may be inserted in the expression cassette
12 in either a sense or antisense orientation.

13 As described in detail in the examples herein, improvements in cold-storage
14 characteristics have been observed in the potato variety Desiree transformed by the methods
15 of the present invention. A direct measure of improved cold-storage characteristics is a
16 reduction in the level of GLTP or GHTP enzyme activity detected in potatoes after harvest
17 and cold-storage. Transformed potato varieties have been developed wherein the total α
18 glucan phosphorylase activity measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers
19 of plants stored at 4°C for 189 days is as much as 70% lower than the total α glucan
20 phosphorylase activity in tubers of untransformed plants stored under the same conditions.

21 Another relatively direct measure of improved cold-storage characteristics is a
22 reduction in sweetening of potatoes observed after a period of cold-storage. Transformed
23 potato varieties have been developed wherein the sum of the concentrations of glucose and
24 fructose in tubers stored at 4°C for 91 days is 39% lower than the sum of the concentrations
25 of glucose and fructose in tubers of an untransformed plant stored under the same conditions.

26 Yet another measure of improved cold-storage characteristics, demonstrating a
27 practical advantage of the present invention, is a reduction in darkening of a potato chip
28 during processing (cooking). As discussed hereinbefore, the accumulation of sugars in
29 potatoes during cold-storage contributes to unacceptable darkening of the fried product.
30 Reduced darkening upon frying can be quantified as a measure of the reflectance, or chip
31 score, of the fried potato chip. Techniques for measuring chip scores are discussed herein.

1 Transformed potato varieties of the present invention have been developed wherein the chip
2 score for tubers of plants stored at 4°C for 124 days was as much as 89% higher than the chip
3 scores for tubers of untransformed plants stored under the same conditions.

4 By reducing GLTP and/or GHTP activity in tubers of potato plants, thereby inhibiting
5 sugar accumulation during cold-temperature storage, the present invention allows for storage
6 of potatoes at cooler temperatures than would be possible with wildtype potatoes of the same
7 cultivar. As discussed above, storage of potatoes at cooler temperatures than those
8 traditionally used could result in increased storage life, increased dormancy through reduced
9 respiration and sprouting, and reduced incidence of disease. It will be apparent to those
10 skilled in the art that such additional benefits also constitute improved cold-storage
11 characteristics and may be measured and quantified by known techniques.

12 13 BRIEF DESCRIPTION OF THE DRAWINGS

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15 In drawings illustrating embodiments of the invention:

16 Figure 1 is a schematic diagram of the tuber L-type α glucan phosphorylase antisense
17 sequence inserted into the pBI121 transformation vector;

18 Figure 2 is a schematic diagram of the tuber H-type α glucan phosphorylase antisense
19 sequence inserted into the pBI121 transformation vector;

20 Figure 3 shows the basic structure of the three isolated isoforms of glucan
21 phosphorylase. The transit peptide (TS) and insertion sequence (IS) are characteristic of the
22 L-type phosphorylases and are not found in the H-type phosphorylase. The percentages
23 indicate the nucleic acid sequence homologies between the isoforms;

24 Figure 4 is a schematic diagram of carbohydrate interconversions in potatoes
25 (Sowokinos 1990);

26 Figure 5 is a comparison of the amino acid sequences of the three isoforms of
27 phosphorylase found in potato for the region targeted by the antisense GLTP construct used in
28 the Examples herein. Highlighted amino acids are identical. The leaf L-type α glucan
29 phosphorylase amino acid sequence is on top (amino acids 21 - 238 of SEQ ID NO: 6), the
30 tuber L-type α glucan phosphorylase amino acid sequence is in the middle (amino acids 49 -

1 266 of SEQ ID NO: 2), and tuber H-type α glucan phosphorylase amino acid sequence is on
2 the bottom (amino acids 46 - 264 of SEQ ID NO: 4);

3 Figure 6A and 6B are a comparison of the nucleotide sequences of the three isoforms
4 of phosphorylase found in potato for the region targeted by the antisense GLTP construct used
5 in the Examples herein. Highlighted nucleotides are identical. The leaf L-type α glucan
6 phosphorylase nucleotide sequence is on top (nucleotides 389 - 1045 of SEQ ID NO: 5), the
7 tuber L-type α glucan phosphorylase nucleotide sequence is in the middle (nucleotides 338 -
8 993 of SEQ ID NO: 1), and tuber H-type α glucan phosphorylase nucleotide sequence is on
9 the bottom (nucleotides 147 - 805 of SEQ ID NO: 3);

10 Figure 7 is a northern blot of polyadenylated RNA isolated from potato tubers of wild
11 type and lines 3,4,5, and 9 transformed with the tuber L-type α glucan phosphorylase. The
12 blot was probed with a radiolabelled probe specific for the tuber L-type α glucan
13 phosphorylase;

14 Figure 8 is a northern blot of total RNA isolated from potato tubers of wild type and
15 lines 1 and 2 transformed with the H-type α -glucan phosphorylase. The blot was probed with
16 a radio labelled probe specific for the H-type α -glucan phosphorylase;

17 Figure 9 shows the fried product obtained from (A) wild type and tuber L-type α
18 glucan phosphorylase transformants (B) ATL1 (C) ATL3 (D) ATL4 (E) ATL5 (F) ATL9 field
19 grown tubers following 86 days storage at 4°C ("ATL" = antisense tuber L-type
20 transformant);

21 Figure 10 shows the activity gel and western blot of L-type and H-type isozymes of α
22 1,4 glucan phosphorylase extracted from wild type tubers and tubers transformed with the
23 antisense construct for the L-type isoform; and

24 Figure 11 shows the activity gel and western blot of L-type and H-type isozymes of α
25 1,4 glucan phosphorylase extracted from wild type tubers and transformed with the antisense
26 construct for the H-type isoform..

27 DESCRIPTION OF THE PREFERRED EMBODIMENT

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29
30 Potato plants having a reduced level of α glucan L-type tuber phosphorylase (GLTP)
31 or α glucan H-type tuber phosphorylase (GHTP) activity in tubers produced by the plants

relative to that of tubers produced by unmodified potato plants are provided. In the exemplified case, reduction in α glucan phosphorylase activity is accomplished by transforming a potato plant with an expression cassette having a plant promoter sequence operably linked to a DNA sequence which, when transcribed in the plant, inhibits expression of an endogenous GLTP gene or GHTP gene. Although, in the exemplified case, the DNA sequence is inserted in the expression cassette in the antisense orientation, a reduction in α glucan phosphorylase activity can be achieved with the DNA sequence inserted in the expression cassette in either a sense or antisense orientation.

1 Homology Dependent Silencing

The control of gene expression using sense or antisense gene fragments is standard laboratory practice and is well documented in the literature. Antisense and sense suppression are both gene sequence homology-dependent phenomena that may be described as "homology-dependent silencing" phenomena.

A review of scientific research articles published during 1996 reveals several hundred reports of homology-dependent silencing in transgenic plants. The mechanisms underlying homology-dependent silencing are not fully understood, but the characteristics of the phenomena have been studied in many plant genes and this body of work has been extensively reviewed (Meyer and Saedler 1996, Matzke and Matzke 1995, Jorgensen 1995, Weintraub 1990, Van der Krol *et al.* 1988). Homology-dependent silencing appears to be a general phenomenon that may be used to control the activity of many endogenous genes. Examples of genes exhibiting reduced expression after the introduction of homologous sequences include dihydroflavanol reductase (Van der Krol 1990), polygalacturonidase (Smith *et al.* 1990), phytoene synthase (Fray and Grierson 1993), pectinesterase (Seymour *et al.* 1993), phenylalanine ammonia-lyase (De Carvalho *et al.* 1992), β -1,3-glucanase (Hart *et al.* 1992), chitinase (Dorlhac *et al.* 1994) nitrate reductase (Napoli *et al.* 1990), and chalcone synthase (14). Transformation of Russet Burbank potato plants with either sense- or antisense- constructs of the potato leafroll virus coat protein gene has been reported to confer resistance to potato leafroll virus infection (Kawchuk *et al.* 1991). The transfer of a homologous sense or antisense sequence usually generates transformants with reduced endogenous gene expression. As discussed in detail in the examples herein, transformed

1 potato plants exhibiting phenotypes indicating reduced GLTP or GHTP expression can be
2 readily identified.

3 In the antisense suppression technique, a gene construct or expression cassette is
4 assembled which, when inserted into a plant cell, results in expression of an RNA which is of
5 complementary sequence to the mRNA produced by the target gene. It is theorized that the
6 complementary RNA sequences form a duplex thereby inhibiting translation to protein. The
7 theory underlying both sense and antisense inhibition has been discussed in the literature,
8 including in *Antisense Research and Applications* (CRC Press, 1993) pp. 125-148. The
9 complementary sequence may be equivalent in length to the whole sequence of the target
10 gene, but a fragment is usually sufficient and is more convenient to work with. For instance,
11 Cannon *et al.* (1990) reveals that an antisense sequence as short as 41 base pairs is sufficient
12 to achieve gene inhibition. United States Patent No. 5,585,545 (Bennett *et al.*, December 17,
13 1996) describes gene inhibition by an antisense sequence of only 20 base pairs. There are
14 many examples in the patent literature of patents including descriptions and claims to
15 methods for suppressing gene expression through the introduction of antisense sequences to
16 an organism, including, for example, United States Patent No. 5,545,815 (Fischer *et al.*,
17 August 13, 1996) and United States Patent No. 5,387,757 (Bridges *et al.*, February 7, 1995).

18 Sense-sequence homology-dependent silencing is conducted in a similar manner to
19 antisense suppression, except that the nucleotide sequence is inserted in the expression
20 cassette in the normal sense orientation. A number of patents, including United States patents
21 5,034,323, 5,231,020 and 5,283,184, disclose the introduction of sense sequences leading to
22 suppression of gene expression.

23 Both forms of homology-dependent silencing, sense- and antisense-suppression, are
24 useful for accomplishing the down-regulation of GLTP or GHTP of the present invention. It
25 is recognized in the art that both techniques are equally useful strategies for gene suppression.
26 For instance, both US Patent No. 5,585,545 (Bennett *et al.*, December 17, 1996) and US
27 Patent No. 5,451,514 (Boudet *et al.*, September 15, 1995) claim methods for inhibiting gene
28 expression or recombinant DNA sequences useful in methods for suppressing gene
29 expression drawn to both sense- and antisense-suppression techniques.

2 Alternate Techniques for Reducing GHTP and/or GLTP Activity in Tubers

Although homology-dependent silencing is a preferred technique for the down-regulation of GLTP or GHTP in potato plants of the present invention, there are several commonly used alternative strategies available to reduce the activity of a specific gene product which will be understood by those skilled in the art to bear application in the present invention. Insertion of a related gene or promoter into a plant can induce rapid turnover of homologous endogenous transcripts, a process referred to as co-suppression and believed to have many similarities to the mechanism responsible for antisense RNA inhibition (Jorgensen, 1995; Brusslan and Tobin, 1995). Various regulatory sequences of DNA can be altered (promoters, polyadenylation signals, post-transcriptional processing sites) or used to alter the expression levels (enhancers and silencers) of a specific mRNA. Another strategy to reduce expression of a gene and its encoded protein is the use of ribozymes designed to specifically cleave the target mRNA rendering it incapable of producing a fully functional protein (Hasseloff and Gerlach, 1988). Identification of naturally occurring alleles or the development of genetically engineered alleles of an enzyme that have been identified to be important in determining a particular trait can alter activity levels and be exploited by classical breeding programs (Ortiz and Huaman, 1994). Site-directed mutagenesis is often used to modify the activity of an identified gene product. The structural coding sequence for a phosphorylase enzyme can be mutagenized in *E. coli* or another suitable host and screened for reduced starch phosphorolysis. Alternatively, naturally occurring alleles of the phosphorylase with reduced affinity and/or specific activity may be identified. Additionally, the activity of a particular enzyme can be altered using various inhibitors. These procedures are routinely used and can be found in text books such as Sambrook *et al.* (1989).

3 Variants of GLTP and GHTP Enzymes and Sequences Used for Homology Dependent Silencing

As discussed in the background of the invention, and in greater detail by Nakano *et al.* (1986), Mori *et al.* (1991), and Sonnewald *et al.* (1990), there are three known α glucan phosphorylase isozymes that occur in potato plants. The present invention relates to down-regulation of the GLTP and/or GHTP isozymes. While it is believed that the GLTP and GHTP genes of all known commercial potato varieties are substantially identical, it is

1 expected that the principles and techniques of the present invention would be effective in
2 potato plants having variant full length polynucleotide sequences or subsequences which
3 encode polypeptides having the starch catabolizing enzymatic activity of the described GLTP
4 and GHTP enzymes. The terms "GLTP" and "GHTP", as used herein and in the claims, are
5 intended to cover the variants described above. The foregoing variants may include GLTP
6 and GHTP nucleotide sequence variants that differ from those exemplified but still encode
7 the same polypeptide due to codon degeneracy, as well as variants which encode proteins
8 capable of recognition by antibodies raised against the GLTP and GHTP amino acid
9 sequences set forth in SEQ ID NO's. 2 and 4.

10 Similarly, those skilled in the art will recognize that homology dependent silencing of
11 GLTP and/or GHTP in potato plants may be accomplished with sense or antisense sequences
12 other than those exemplified. First, the region of the GLTP or GHTP cDNA sequence from
13 which the antisense sequence is derived is not essential. Second, as described hereinabove,
14 the length of the antisense sequence used may vary considerably. Further, the sense or
15 antisense sequence need not be identical to that of the target GLTP or GHTP gene to be
16 suppressed. As described in the Examples herein, the inventors have observed that
17 transformation of potato plants with antisense DNA sequences derived from the GHTP gene
18 not only substantially suppresses GHTP gene activity, but causes some degree of suppression
19 of GLTP gene activity. The GHTP and GLTP genes antisense sequences have 56.8%
20 sequence identity. The sequence identity between the GLTP antisense sequence and the
21 corresponding leaf type α glucan phosphorylase sequence described by Sonnewald *et al.*
22 (1990) is 71.3%. In the inventors' research to date, the same phenomenon of cross-
23 downregulation has not been observed when potato plants are transformed with antisense
24 DNA sequences derived from the GLTP gene. Nevertheless, these results clearly indicate that
25 absolute sequence identity between the target endogenous α glucan phosphorylase gene and
26 the recombinant DNA is not essential given that GLTP activity was suppressed by an
27 antisense sequence having about 57% sequence identity with the target GLTP sequence.

28 Thus, it will be understood by those skilled in the art that sense or antisense sequences
29 other than those exemplified herein and other than those having absolute sequence identity
30 with the target endogenous GLTP or GHTP gene will be effective to cause suppression of the
31 endogenous GLTP or GHTP gene when introduced into potato plant cells. Useful sense or

1 antisense sequences may differ from the exemplified antisense sequences or from other
2 sequences derived from the endogenous GHTP or GLTP gene sequences by way of
3 conservative amino acid substitutions or differences in the percentage of matched nucleotides
4 or amino acids over portions of the sequences which are aligned for comparison purposes.

5 United States Patent 5,585,545 (Bennett *et al.*, December 17, 1996) provides a helpful
6 discussion regarding techniques for comparing sequence identity for polynucleotides and
7 polypeptides, conservative amino acid substitutions, and hybridization conditions indicative
8 of degrees of sequence identity. Relevant parts of that discussion are summarized herein.

9 Percentage of sequence identity for polynucleotides and polypeptides may be
10 determined by comparing two optimally aligned sequences over a comparison window,
11 wherein the portion of the polynucleotide or polypeptide sequence in the comparison window
12 may include additions or deletions (i.e., gaps) as compared to the reference sequence (which
13 does not comprise additions or deletions) for optimal alignment of the two sequences. The
14 percentage is calculated by: (a) determining the number of positions at which the identical
15 nucleic acid base or amino acid residue occurs in both sequences to yield the number of
16 matched positions; (b) dividing the number of matched positions by the total number of
17 positions in the window of comparison; and, (c) multiplying the result by 100 to yield the
18 percentage of sequence identity. Optimal alignment of sequences for comparison may be
19 conducted by computerized implementations of known algorithms (e.g., GAP, BESTFIT,
20 FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer
21 Group (GCG), 575 Science Dr., Madison, WI, or BlastN and BlastX available from the
22 National Center for Biotechnology Information), or by inspection.

23 Polypeptides which are substantially similar share sequences as noted above
24 except that residue positions which are not identical may differ by conservative amino acid
25 changes. Conservative amino acid substitutions refer to the interchangeability of residues
26 having similar side chains. For example, a group of amino acids having aliphatic side chains
27 is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having
28 aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having
29 amide-containing side chains is asparagine and glutamine; a group of amino acids having
30 aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids

1 having basic side chains is lysine, arginine, and histidine; and a group of amino acids having
2 sulfur-containing side chains is cysteine and methionine.

3 Another indication that nucleotide sequences are substantially identical is if two
4 molecules specifically hybridize to each other under stringent conditions. Stringent
5 conditions are sequence dependent and will be different in different circumstances.
6 Generally, stringent conditions are selected to be about 10°C lower than the thermal melting
7 point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the
8 temperature (under defined ionic strength and pH) at which 50% of the target sequence
9 hybridizes to a perfectly matched probe. The T_m of a hybrid, which is a function of both the
10 length and the base composition of the probe, can be calculated as described in Sambrook *et*
11 *al.* (1989). Typically, stringent conditions for a Southern blot protocol involve washing at
12 65°C with 0.2XSSC. For preferred oligonucleotide probes, washing conditions are typically
13 about at 42°C in 6XSSC.

14 15 4 General Methods

16 Various methods are available to introduce and express foreign DNA sequences in
17 plant cells. In brief, the steps involved in preparing antisense α glucan phosphorylase cDNAs
18 and introducing them into a plant cell include: (1) isolating mRNA from potato plants and
19 preparing cDNA from the mRNA; (2) screening the cDNA for the desired sequences; (3)
20 linking a promoter to the desired cDNAs in the opposite orientation for expression of the
21 phosphorylase genes; (4) transforming suitable host plant cells; and (5) selecting and
22 regenerating cells which transcribe the inverted sequences.

23 In the exemplified case, DNA derived from potato GLTP and GHTP genes is used to
24 create expression cassettes having a plant promoter sequence operably linked to an antisense
25 DNA sequence which, when transcribed in the plant, inhibits expression of an endogenous
26 GLTP gene or GHTP gene. *Agrobacterium tumefaciens* is used as a vehicle for transmission
27 of the DNA to stem explants of potato plant shoots. A plant regenerated from the
28 transformed explants transcribes the antisense DNA which inhibits activity of the enzyme.

29 The recombinant DNA technology described herein involves standard laboratory
30 techniques that are well known in the art and are described in standard references such as
31 Sambrook *et al.* (1989). Generally, enzymatic reactions involving DNA ligase, DNA

polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications.

5 Preparation of GHTP and GLTP cDNA

cDNA is prepared from isolated potato tuber mRNA by reverse transcription. A primer is annealed to the mRNA, providing a free 3' end that can be used for extension by the enzyme reverse transcriptase. The enzyme engages in the usual 5'-3' elongation, as directed by complementary base pairing with the mRNA template to form a hybrid molecule, consisting of a template RNA strand base-paired with the complementary cDNA strand. After degradation of the original mRNA, a DNA polymerase is used to synthesize the complementary DNA strand to convert the single-stranded cDNA into a duplex DNA.

After DNA amplification, the double stranded cDNA is inserted into a vector for propagation in *E. coli*. Typically, identification of clones harbouring the desired cDNA's would be performed by either nucleic acid hybridization or immunological detection of the encoded protein, if an expression vector is used. In the exemplified case, the matter is simplified in that the DNA sequences of the GLTP and GHTP genes are known, as are the sequences of suitable primers (Brisson *et al.*, 1990; Fukui *et al.*, 1991). The primers used hybridize within the GLTP and GHTP genes. Thus, it is expected that the amplified cDNA's prepared represent portions of the GLTP and GHTP genes without further analysis. *E. coli* transformed with pUC19 plasmids carrying the phosphorylase DNA insert were detected by color selection. Appropriate *E. coli* strains transformed with plasmids which do not carry inserts grow as blue colonies. Strains transformed with pBluescript plasmids carrying inserts grow as white colonies. Plasmids isolated from transformed *E. coli* were sequenced to confirm the sequence of the phosphorylase inserts.

6 Vector Construction

The cDNAs prepared can be inserted in the antisense or sense orientation into expression cassette in expression vectors for transformation of potato plants to inhibit the expression of the GLTP and/or GHTP genes in potato tubers.

As in the exemplified case, which involves antisense suppression, the desired recombinant vector will comprise an expression cassette designed for initiating transcription

1 of the antisense cDNAs in plants. Additional sequences are included to allow the vector to be
2 cloned in a bacterial or phage host.

3 The vector will preferably contain a prokaryote origin of replication having a broad
4 host range. A selectable marker should also be included to allow selection of bacterial cells
5 bearing the desired construct. Suitable prokaryotic selectable markers include resistance to
6 antibiotics such as ampicillin.

7 Other DNA sequences encoding additional functions may also be present in the
8 vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations,
9 T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

10 For expression in plants, the recombinant expression cassette will contain in
11 addition to the desired sequence, a plant promoter region, a transcription initiation site (if
12 the sequence to be transcribed lacks one), and a transcription termination sequence. Unique
13 restriction enzyme sites at the 5' and 3' ends of the cassette are typically included to allow for
14 easy insertion into a pre-existing vector. Sequences controlling eukaryotic gene
15 expression are well known in the art.

16 Transcription of DNA into mRNA is regulated by a region of DNA referred to as the
17 promoter. The promoter region contains sequence of bases that signals RNA polymerase to
18 associate with the DNA, and to initiate the transcription of mRNA using one of the DNA
19 strands as a template to make a corresponding complementary strand of RNA. Promoter
20 sequence elements include the TATA box consensus sequence (TATAAT), which is usually
21 20 to 30 base pairs (bp) upstream (by convention -30 to -20 bp relative to the transcription
22 start site) of the transcription start site. In most instances the TATA box is required for
23 accurate transcription initiation. The TATA box is the only upstream promoter element that
24 has a relatively fixed location with respect to the start point.

25 The CAAT box consensus sequence is centered at -75, but can function at distances
26 that vary considerably from the start point and in either orientation.

27 Another common promoter element is the GC box at -90 which contains the
28 consensus sequence GGGCGG. It may occur in multiple copies and in either orientation.

29 Other sequences conferring tissue specificity, response to environmental signals, or
30 maximum efficiency of transcription may also be found in the promoter region. Such
31 sequences are often found within 400 bp of transcription initiation size, but may extend as far

1 as 2000 bp or more. In heterologous promoter/structural gene combinations, the promoter is
2 preferably positioned about the same distance from the heterologous transcription start site as
3 it is from the transcription start site in its natural setting. However, some variation in this
4 distance can be accommodated without loss of promoter function.

5 The particular promoter used in the expression cassette is not critical to the
6 invention. Any of a number of promoters which direct transcription in plant cells is suitable.
7 The promoter can be either constitutive or inducible.

8 A number of promoters which are active in plant cells have been described in the
9 literature. These include the nopaline synthase (NOS) and octopine synthase (OCS)
10 promoters (which are carried on tumour-inducing plasmids of *Agrobacterium tumefaciens*),
11 the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S and 35S and
12 the figwort mosaic virus 35S-promoters, the light-inducible promoter from the small subunit
13 of ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide),
14 and the chlorophyll a/b binding protein gene promoter, etc. All of these promoters have been
15 used to create various types of DNA constructs which have been expressed in plants; see, e.g.,
16 PCT WO8402913.

17 The CaMV 35S promoter used in the Examples herein, has been shown to be highly
18 active and constitutively expressed in most tissues (Bevan *et al.*, 1986). A number of other
19 genes with tuber-specific or enhanced expression are known, including the potato tuber
20 ADPGPP genes, large and small subunits (Muller *et al.*, 1990). Other promoters which are
21 contemplated to be useful in this invention include those that show enhanced or specific
22 expression in potato tubers, that are promoters normally associated with the expression of
23 starch biosynthetic or modification enzyme genes, or that show different patterns of
24 expression, for example, or are expressed at different times during tuber development.
25 Examples of these promoters include those for the genes for the granule-bound and other
26 starch synthases, the branching enzymes (Blennow *et al.*, 1991; WO 9214827; WO 9211375),
27 disproportionating enzyme (Takaha *et al.*, 1993) debranching enzymes, amylases, starch
28 phosphorylases (Nakano *et al.*, 1989; Mori *et al.*, 1991), pectin esterases (Ebbelaar *et al.*,
29 1993), the 40 kD glycoprotein; ubiquitin, aspartic proteinase inhibitor (Stukerlj *et al.*, 1990),
30 the carboxypeptidase inhibitor, tuber polyphenol oxidases (Shahar *et al.*, 1992; GenBank
31 Accession Numbers M95196 and M95197), putative trypsin inhibitor and other tuber cDNAs

(Stiekema *et al.*, 1988), and for amylases and sporamins (Yoshida *et al.*, 1992; Ohta *et al.*, 1991).

In addition to a promoter sequence, the expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. In the exemplified case the nopaline synthase NOS 3' terminator sequence (Bevan *et al.* 1983) was used.

Polyadenylation sequences are also commonly added to the vector construct if the mRNA encoded by the structural gene is to be efficiently translated (Alber and Kawasaki, 1982). Polyadenylation is believed to have an effect on stabilizing mRNAs. Polyadenylation sequences include, but are not limited to the *Agrobacterium* octopine synthase signal (Gielen *et al.*, 1984) or the nopaline synthase signal (Depicker *et al.*, 1982).

The vector will also typically contain a selectable marker gene by which transformed plant cells can be identified in culture. Typically, the marker gene encodes antibiotic resistance. These markers include resistance to G418, hygromycin, bleomycin, kanamycin, and gentamycin. In the exemplified case, the marker gene confers resistance to kanamycin. After transforming the plant cells, those cells containing the vector will be identified by their ability to grow in a medium containing the particular antibiotic.

7 Transformation of Plant Cells

Although in the exemplified case potato plant shoot stem explants were transformed via inoculation with *Agrobacterium tumefaciens* carrying the antisense sequence linked to a binary vector, direct transformation techniques which are known in the art can also be used to transfer the recombinant DNA. The vector can be microinjected directly into plant cells. Alternatively, nucleic acids may be introduced to the plant cell by high velocity ballistic penetration by small particles having the nucleic acid of interest embedded within the matrix of the particles or on the surface. Fusion of protoplasts with lipid-surfaced bodies such as minicells, cells or lysosomes carrying the DNA of interest can be used. The DNA may also be introduced into plant cells by electroporation, wherein plant protoplasts are electroporated in the presence of plasmids carrying the expression cassette.

1 In contrast to direct transformation methods, the exemplified case uses vectored
2 transformation using *Agrobacterium tumefaciens*. *Agrobacterium tumefaciens* is a Gram-
3 negative soil bacteria which causes a neoplastic disease known as crown gall in
4 dicotyledonous plants. Induction of tumours is caused by tumour-inducing plasmids known
5 as Ti plasmids. Ti plasmids direct the synthesis of opines in the infected plant. The opines
6 are used as a source of carbon and/or nitrogen by the Agrobacteria.

7 The bacterium does not enter the plant cell, but transfers only part of the Ti plasmid, a
8 portion called T-DNA, which is stably integrated into the plant genome, where it expresses
9 the functions needed to synthesize opines and to transform the plant cell. *Vir* (virulence)
10 genes on the Ti plasmid, outside of the T-DNA region, are necessary for the transfer of the T-
11 DNA. The *vir* region, however, is not transferred. In fact, the *vir* region, although required
12 for T-DNA transfer, need not be physically linked to the T-DNA and may be provided on a
13 separate plasmid.

14 The tumour-inducing portions of the T-DNA can be interrupted or deleted without
15 loss of the transfer and integration functions, such that normal and healthy transformed plant
16 cells may be produced which have lost all properties of tumour cells, but still harbour and
17 express certain parts of T-DNA, particularly the T-DNA border regions. Therefore, modified
18 Ti plasmids, in which the disease causing genes have been deleted, may be used as vectors for
19 the transfer of the sense and antisense gene constructs of the present invention into potato
20 plants (see generally Winnacker, 1987).

21 Transformation of plants cells with *Agrobacterium* and regeneration of whole plants
22 typically involves either co-cultivation of *Agrobacterium* with cultured isolated protoplasts or
23 transformation of intact cells or tissues with *Agrobacterium*. In the exemplified case, stem
24 explants from potato shoot cultures are transformed with *Agrobacterium*.

25 Alternatively, cauliflower mosaic virus (CaMV) may be used as a vector for
26 introducing sense or antisense DNA into plants of the *Solanaceae* family. For instance,
27 United States Patent No. 4,407,956 (Howell, October 4, 1983) teaches the use of cauliflower
28 mosaic virus DNA as a plant vehicle.

29

8 Selection and Regeneration of Transformed Plant Cells

After transformation, transformed plant cells or plants carrying the antisense or sense DNA must be identified. A selectable marker, such as antibiotic resistance, is typically used. In the exemplified case, transformed plant cells were selected by growing the cells on growth medium containing kanamycin. Other selectable markers will be apparent to those skilled in the art. For instance, the presence of opines can be used to identify transformants if the plants are transformed with *Agrobacterium*.

Expression of the foreign DNA can be confirmed by detection of RNA encoded by the inserted DNA using well known methods such as Northern blot hybridization. The inserted DNA sequence can itself be identified by Southern blot hybridization or the polymerase chain reaction, as well (See, generally, Sambrook *et al.* (1989)).

Generally, after it is determined that the transformed plant cells carry the recombinant DNA, whole plants are regenerated. In the exemplified case, stem and leaf explants of potato shoot cultures were inoculated with a culture of *Agrobacterium tumefaciens* carrying the desired antisense DNA and a kanamycin marker gene. Transformants were selected on a kanamycin-containing growth medium. After transfer to a suitable medium for shoot induction, shoots were transferred to a medium suitable for rooting. Plants were then transferred to soil and hardened off. The plants regenerated in culture were transplanted and grown to maturity under greenhouse conditions.

9 Analysis of GHTP and GLTP Activity Levels in Transformed Tubers

Following regeneration of potato plants transformed with antisense DNA sequences derived from the GHTP and GLTP genes, the biochemistry of transformed tuber tissue was analyzed several ways. The *in vitro* activity of α glucan phosphorylase in the phosphorolytic direction was assayed according to the methods of Steup (1990) (Table 1). The activity of the enzyme in the synthetic direction and the amount of enzyme protein were compared after electrophoretic separation of the enzyme isoforms on a glycogen-containing, polyacrylamide gel (Figure 7). Starch synthesis by the tuber L-type and H-type isoforms was determined by iodine staining of the gel after incubation with glucose-1-phosphate and a starch primer (Steup, 1990). Western analysis was performed by blotting the protein from an identical unincubated native gel to nitrocellulose and probing with polyclonal antibodies specific for

tuber type L and type H glucan phosphorylase isoforms. Levels of reducing sugars (glucose and fructose) in tuber tissues were quantified by HPLC (Tables 2, 3 and 4). The extent of Maillard reaction, which is proportional to the concentration of reducing sugars in tubers was examined by determining chip scores after frying (Table 5 and Figure 6).

10 Definitions

As used herein and in the claims, the term:

- "about three months", "about four months" and "about six months" refer, respectively, to periods of time of three months plus or minus two weeks, four months plus or minus two weeks, and six months plus or minus two weeks;
- "antisense orientation" refers to the orientation of nucleic acid sequence from a structural gene that is inserted in an expression cassette in an inverted manner with respect to its naturally occurring orientation. When the sequence is double stranded, the strand that is the template strand in the naturally occurring orientation becomes the coding strand, and vice versa;
- "chip score" of a tuber means the reflectance measurement recorded by an Agtron model E-15-FP Direct Reading Abridged Spectrophotometer (Agtron Inc. 1095 Spice Island Drive #100, Sparks Nevada 89431) of a center cut potato chip fried at 205°F in soybean oil for approximately 3 minutes until bubbling stops;
- "cold storage" or "storage at reduced temperature" or variants thereof, shall mean holding at temperatures less than 10°C, that may be achieved by refrigeration or ambient temperatures;
- "endogenous", as it is used with reference to α glucan phosphorylase genes of a potato plant, shall mean a naturally occurring gene that was present in the genome of the potato plant prior to the introduction of an expression cassette carrying a DNA sequence derived from an α glucan phosphorylase gene;
- "expression" refers to the transcription and translation of a structural gene so that a protein is synthesized;
- "heterologous sequence" or "heterologous expression cassette" is one that originates from a foreign species, or, if from the same species, is substantially modified from its original form;

- 1 - "improved cold-storage characteristics" includes, without limitation, improvements in
2 chip score and reduction in sugar accumulation in tubers measured at harvest or after a period
3 of storage below 10°C, and further includes improvements, advantages and benefits which
4 may result from the storage of potatoes at cooler temperatures than those traditionally used,
5 such as, without limitation, increased storage life of potatoes, increased dormancy through
6 reduced respiration and sprouting of potatoes, and reduced incidence of disease. Unless
7 further qualified by a specific measure or test, an improvement in a cold-storage characteristic
8 refers to a difference in the described characteristic relative to that in a control, wildtype or
9 unmodified potato plant;
- 10 - "modified" or variants thereof, when used to describe potato plants or tubers, is used
11 to distinguish a potato plant or tuber that has been altered from its naturally occurring state
12 through: the introduction of a nucleotide sequence from the same or a different species,
13 whether in a sense or antisense orientation, whether by recombinant DNA technology or by
14 traditional cross-breeding methods including the introduction of modified structural or
15 regulatory sequences; modification of a native nucleotide sequence by site-directed
16 mutagenesis or otherwise; or the treatment of the potato plant with chemical or protein
17 inhibitors. An "unmodified" potato plant or tuber means a control, wildtype or naturally
18 occurring potato plant or tuber that has not been modified as described above;
- 19 - "nucleic acid sequence" or "nucleic acid segment" refer to a single or double-stranded
20 polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It
21 includes both self-replicating plasmids, infectious polymers of DNA or RNA and non-
22 functional DNA or RNA;
- 23 - "operably linked" refers to functional linkage between a promoter and a second
24 sequence, wherein the promoter sequence initiates transcription of RNA corresponding to the
25 second sequence;
- 26 - "plant " includes whole plants, plant organs (e.g. leaves, stems, roots, etc.) seeds and
27 plant cells;
- 28 - "promoter" refers to a region of DNA upstream from the structural gene and involved
29 in recognition and binding RNA polymerase and other proteins that initiate transcription. A
30 "plant promoter" is a promoter capable of initiating transcription in plant cells;

- 1 - "reduced activity" or variants thereof, when used in reference to the level of GLTP or
2 GLTP enzyme activity in a potato tuber includes reduction of GLTP or GHTP enzyme
3 activity resulting from reduced expression of the GLTP or GHTP gene product, reduced
4 substrate affinity of the GLTP or GHTP enzyme, and reduced catalytic activity of the GLTP
5 or GHTP enzyme;
- 6 - "reduced" or variants thereof, may be used herein with reference to, without
7 limitation, activity levels of GLTP or GHTP enzyme in potato tubers, accumulation of sugars
8 in potato tubers and darkening of potato chips upon frying. Unless further qualified by a
9 specific measure or test, reduced levels or reduced activity refers to a demonstrable
10 statistically significant difference in the described characteristic relative to that in a control,
11 wildtype or unmodified potato plant;
- 12 - "stress" or variants thereof, when used in relation to stresses experienced by potato
13 plants and tubers, includes the effects of environment, fertility, moisture, temperature,
14 handling, disease, atmosphere and aging that impact upon plant or tuber quality and which
15 may be experienced by potato plants through all stages of their life cycle and by tubers at all
16 stages of the growth and development cycle and during subsequent harvesting, transport,
17 storage and processing;
- 18 - "stress resistance" or variants thereof, shall mean reduced effects of temperature,
19 aging, disease, atmosphere, physical handling, moisture, chemical residues, environment,
20 pests and other stresses;
- 21 - "suitable host" refers to a microorganism or cell that is compatible with a recombinant
22 plasmid, DNA sequence or recombinant expression cassette and will permit the plasmid to
23 replicate, to be incorporated into its genome, or to be expressed; and
- 24 - "uninterrupted" refers to a DNA sequence (e.g. cDNA) containing an open reading
25 frame that lacks intervening, untranslated sequences.

EXAMPLE 1

28 This example describes the reduction of GHTP and/or GLTP activity in tubers of
29 potato plants by transforming potato plants with expression cassettes containing DNA
30 sequences derived from the GLTP and GHTP gene sequences linked to the promoter in the
31 antisense orientation.

1 A Isolation of Potato Tuber mRNA

2 Potato total RNA was purified at 4°C using autoclaved reagents from 1g of tuber
3 tissue ground to a fine powder under liquid nitrogen with a mortar and pestle. The powder
4 was transferred to a 30ml corex tube and 3 volumes were added of 100 mM Tris-Cl, pH 8.0,
5 100 mM NaCl, and 10 mM EDTA (10x TNE) containing 0.2% (w/v) SDS and 0.5% (v/v) 2-
6 mercaptoethanol. An equal volume of phenol-chloroform (1:1) was added and the sample
7 gently vortexed before centrifugation at 4 °C in a SS34 rotor at 8,000 rpm for 5 min. The
8 organic phase was reextracted with 0.5 volume of 10x TNE containing 0.2% (w/v)SDS and
9 0.5% (v/v) 2-mercaptoethanol and the combined aqueous phases were extracted with
10 chloroform. Nucleic acids were precipitated from the aqueous phase with sodium acetate and
11 absolute ethanol, pelleted by centrifugation, and resuspended in 3 ml of 1x TNE. An equal
12 volume of 5 M LiCl was added and the sample stored at -20°C for at 4 h before centrifuging
13 at 8,000 rpm in a SS34 rotor at 4°C for 10 min. The RNA pellet was washed with 70%
14 ethanol, dried, and resuspended in DEPC-treated water.

15 Poly (A⁺) RNA was isolated using oligo (dT) cellulose (Boehringer Mannheim)
16 column chromatography. Poly (A⁺) RNA was isolated from total RNA resuspended in
17 RNase free water. Columns were prepared using an autoclaved Bio-Rad Poly-Prep 10 ml
18 column to which was added 50 mg of oligo (dT) cellulose suspended in 1 ml of loading buffer
19 B which contains 20 mM Tris-Cl, pH 7.4, 0.1 M NaCl, 1 mM EDTA, and 0.1% (w/v) SDS.
20 The column was washed with 3 volumes of 0.1 M NaOH with 5 mM EDTA and then DEPC-
21 treated water until the pH of effluent was less than 8, as determined with pH paper. The
22 column was then washed with 5 volumes of loading buffer A containing 40 mM Tris-Cl, pH
23 7.4, 1 M NaCl, 1 mM EDTA, and 0.1% (w/v) SDS.

24 RNA samples were heated to 65°C for 5 min at which time 400 µl of loading buffer
25 A, prewarmed to 65°C, was added. The sample was mixed and allowed to cool at room
26 temperature for 2 min before application to the column. Eluate was collected, heated to 65°C
27 for 5 min, cooled to room temperature for 2 min, and reapplied to the column. This was
28 followed by a 5 volume washing with loading buffer A followed by a 4 volume wash with
29 loading buffer B. Poly (A⁺) RNA was eluted with 3 volumes of 10 mM Tris-Cl, pH 7.4, 1
30 mM EDTA, and 0.05% (w/v) SDS. Fractions were collected and those containing RNA were
31 identified in an ethidium bromide plate assay, a petri dish with 1% agarose made with TAE

1 containing EtBr. RNA was precipitated, resuspended in 10 μ l, and a 1 μ l aliquot quantitated
2 with a spectrophotometer.

3 4 B Isolation of GLTP and GHTP DNA Sequences

5 The nucleotide sequences utilized in the development of the antisense construct were
6 randomly selected from the 5' sequence of GLTP (SEQ ID NO: 1) and GHTP (SEQ ID NO:
7 3). DNA sequences used to develop the antisense constructs were obtained using reverse
8 transcription-polymerase chain reaction. GLTP (SPL1 and SPL2)- and GHTP (SPH1 and
9 SPH2)-specific primers were designed according to the published sequences (Brisson et al.
10 1990, Fukui et al. 1991) with minor modifications to facilitate restriction with enzymes:

11 SPL1 Primer: 5'ATTCGAAAAGCTCGAGATTTGCATAGA3' (SEQ ID NO: 7) (additional
12 CG creates Xho I site);

13 SPL2 Primer: 5'GTGTGCTCTCGAGCATTGAAAGC3' (SEQ ID NO: 8) (changed C to G to
14 create Xho I site);

15 SPH1 Primer: 5'GTTTATTTTCCATCGATGGAAGGTGGTG3' (SEQ ID NO: 9) (added
16 CGAT to create Cla I site);

17 SPH2 Primer: 5'ATAATATCCTGAATCGATGCACTGC3' (SEQ ID NO: 10) (changed G to
18 T to create Cla I site).

19 Reverse transcription was performed in a volume of 15 μ l containing 1 x PCR buffer
20 (10 mM Tris-Cl pH 8.2, 50 mM KCl, 0.001% gelatin, 1.5 mM $MgCl_2$), 670 μ M of each
21 dNTP, 6 μ g of total potato tuber cv. Russet Burbank RNA, 1 mM each primer (SPH1 and
22 SPL2, or SPH1 and SPH2) and 200 U of Maloney murine leukemia virus reverse
23 transcriptase (BRL). The reaction was set at 37°C for 30 minutes, then heat-killed at 94°C for
24 5 minutes and snap cooled on ice. To the reverse transcription reaction was added 2.5 U Taq
25 DNA polymerase (BRL) in 35 μ l of 1 x PCR buffer. DNA amplification was done in a Perkin
26 Elmer 480 programmed for 30 cycles with a 1 min 94°C denaturation step, a 1 min 56 °C
27 (SPL1 and SPL2) or 58°C (SPH1 and SPH2) annealing step, and a 2 min 72°C extension
28 step. PCR was completed with a final 10 min extension at 72°C.

29

C Construction of SP Vectors for Phosphorylase Inhibition

To express the antisense constructs in plant cells, it was necessary to fuse the genes to the proper plant regulatory regions. This was accomplished by cloning the antisense DNA into a plasmid vector that contained the needed sequences.

Amplified DNA was blunt ended and cloned into a pUC19 vector at the *Sma*I site. The recombinant plasmid was transformed into sub-cloning efficiency *E. coli* DH5 α cells (BRL). The transformed cells were plated on LB (15 g/l Bactotryptone, 5 g/l yeast extract, 10 g/l NaCl, pH 7.3, and solidified with 1.5% agar) plates that contained ampicillin at 100 ug/ml. Selection of bacteria containing plasmids with inserted plant phosphorylase sequence was accomplished using color selection. The polylinker and T3 and T7 RNA polymerase promoter sequences are present in the N-terminal portion of the *lacZ* gene fragment. pUC19 plasmids without inserts in the polylinker grow as blue colonies in appropriate bacterial strains such as DH5 α . Color selection was made by spreading 100 μ l of 2% X-gal (prepared in dimethyl formamide) on LB plates containing 50 μ g/ml ampicillin 30 minutes prior to plating the transformants. Colonies containing plasmids without inserts will be blue after incubation for 12 to 18 hours at 37C and colonies with plasmids containing inserts will remain white. An isolated plasmid was sequenced to confirm the sequence of the phosphorylase inserts. Sequences were determined using the ABI Prism Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems, Foster City, CA), M13 universal and reverse primers, and an ABI automated DNA sequencer. The engineered plasmid was purified by the rapid alkaline extraction procedure from a 5 ml overnight culture (Birnboim and Doly, 1979). Orientation of the SPL and SPH fragments in pUC19 was determined by restriction enzyme digestion. The recombinant pUC19 vectors and the binary vector pBI121 (Clontech) were restricted, run on a agarose gel and the fragments purified by gel separation as described by Thuring et al (1975).

Ligation fused the antisense sequence to the binary vector pBI121. The ligation contained pBI121 vector that had been digested with *Bam*HI and *Sac*I, along with the SPL or SPH phosphorylase DNA product, that had been cut from the pUC19 subclone with *Bam*HI and *Sac*I. Ligated DNA was transformed into SCE *E. coli* DH5 α cells, and the transformed cells were plated on LB plates containing ampicillin. The nucleotide sequences of the antisense DNA SPL and SPH are nucleotides 338 to 993 of SEQ ID NO: 1 and nucleotides

147 to 799 of SEQ ID NO: 3, respectively. Selection of pBI121 with phosphorlylase inserts was done with CAMV and NOS specific primers.

Samples 1 and 2 representing the tuber L-type and tuber H-type phosphorylase DNA fragments were picked from a plate after overnight growth. These samples were inoculated into 5 ml of LB media and grown overnight at 37°C. Plasmids were isolated by the rapid alkaline extraction procedure, and the DNA was electroporated into *Agrobacterium tumefaciens*.

Constructs were engineered into the pBI121 vector that contains the CaMV 35S promoter (Kay et al. 1987) and the NOS 3' terminator (Bevan et al. 1983) sequence. The pBI121 plasmid is made up of the following well characterized segments of DNA. A 0.93 kb fragment isolated from transposon Tn7 which encodes bacterial spectinomycin/streptomycin (Spc/Str) resistance and is a determinant for selection in *E. coli* and *Agrobacterium tumefaciens* (Fling et al., 1985). This is joined to a chimeric kanamycin resistance gene engineered for plant expression to allow selection of the transformed tissue. The chimeric gene consists of the 0.35 kb cauliflower mosaic virus 35S promoter (P-35S) (Odell et al., 1985), the 0.83 kb neomycin phosphotransferase type II gene (NPTII), and the 0.26 kb 3' non-translated region of the nopaline synthase gene (NOS 3') (Fraley et al., 1983). The next segment is a 0.75 kb origin of replication from the RK2 plasmid (ori-V) (Stalker et al., 1981). It is joined to a 3.1 kb *SalI* to *PvuI* segment of pBR322 which provides the origin of replication for maintenance in *E. coli* (ori-322) and the *bom* site for the conjugational transfer in the *Agrobacterium tumefaciens* cells. Next is a 0.36 kb *PvuI* fragment from the pTiT37 plasmid which contains the nopaline-type T-DNA right border region (Fraley et al., 1985). The antisense sequence was engineered for expression in the tuber by placing the gene under the control of a constitutive tissue non-specific promoter.

D Plant Transformation/Regeneration

The SPL and SPH vectors were transformed into the Desiree potato cultivar according to de Block (1988). To transform "Desiree" potatoes, sterile shoot cultures of "Desiree" were maintained in test tubes containing 8 ml of S1 (Murashige and Skoog (MS) medium supplemented with 2% sucrose and 0.5 g/l MES pH 5.7, solidified with 6 g/l Phytagar). When plantlets reached approximately 5 cm in length, leaf pieces were excised with a single cut

1 along the base and inoculated with a 1:10 dilution of an overnight culture of Agrobacterium
2 tumefaciens. The stem explants were co-cultured for 2 days at 20°C on S1 medium (De
3 Block 1988). Following co-culture, the explants were transferred to S4 medium (MS medium
4 without sucrose, supplemented with 0.5 g/l MES pH 5.7, 200 mg/l glutamine, 0.5 g/l PVP, 20
5 g/l mannitol, 20 g/l glucose, 40 mg/l adenine, 1 mg/l trans zeatin, 0.1 mg/l NAA, 1 g/l
6 carbenicillin, 50 mg/l kanamycin, solidified with 6 g/l phytagar) for 1 week and then 2 weeks
7 to induce callus formation.

8 After 3 weeks, the explants were transferred to S6 medium (S4 without NAA and with
9 half the concentration (500 mg/l) of carbenicillin). After another two weeks, the explants
10 were transferred to S8 medium (S6 with only 250 mg/l carbenicillin and 0.01 mg/l gibberellic
11 acid, GA3) to promote shoot formation. Shoots began to develop approximately 2 weeks
12 after transfer to S8 shoot induction medium. These shoots were excised and transferred to
13 vials of S1 medium for rooting. After about 6 weeks of multiplication on the rooting
14 medium, the plants were transferred to soil and are gradually hardened off.

15 Desiree plants regenerated in culture were transplanted in 1 gallon pots and were
16 grown to maturity under greenhouse conditions. Tubers were harvested and allowed to
17 suberize at room temperature for two days. All tubers greater than 2 cm in length were
18 collected and stored at 4°C under high humidity.

20 E Field Trials

21 Untransformed controls, plants expressing the SPL construct, and plants expressing
22 the SPH construct were propagated in field trials in a single replicate randomized design. All
23 plants were grown side by side in the same field and exposed to similar pesticide, fertilizer,
24 and irrigation regimes. Tubers were harvested and stored at 10°C for 2 weeks before
25 randomly selecting a fraction of the tubers from each line to be placed in storage at 4°C.

27 F Sugar Analysis

28 Tubers were stored at 4°C and were not allowed to recondition at room temperature
29 prior to sugar analysis. An intact longitudinal slice (1 cm thick, width variable and equal to
30 the outside dimensions of the tuber) was cut from the central portion of each tuber, thus
31 representing all of the tuber's tissues. At each harvest, the central slices from four tubers per

clone (3 replicates) were collectively diced into 1-cm cubes and 15 g was randomly selected from the pooled tissue for analysis. Glucan phosphorylase (see below) and sugars were extracted with 15 mL of Tris buffer (50 mM, pH 7.0) containing 2 mM sodium bisulfite, 2 mM EDTA, 0.5 mM PMSF and 10% (w/w) glycerol with a polytron homogenizer at 4°C. The extracts were centrifuged at 4°C (30,000 g, 30 min) and reducing sugars (glucose and fructose) were measured on a 10-fold dilution of the supernatant using a Spectra Physics high performance liquid chromatograph interfaced to a refractive index detector. The separation was performed at 80°C on a 30 x 0.78 cm Aminex HPX 87C column (Biorad) using 0.6 ml/min water as the mobile phase. Calibration of the instrument was via authentic standards of d-glucose and d-fructose.

G Analysis of α -Glucan Phosphorylase Activity

Tubers stored at 4°C were not allowed to warm prior to extraction and analysis of α glucan phosphorylase activity and isozymes. The *in vitro* activity of glucan phosphorylase in the phosphorolytic direction was assayed as described by Steup (1990). Briefly, samples of extracts obtained for sugar analysis (see above) were added to a reaction medium which coupled starch phosphorolysis to the reduction of NADP through the sequential actions of phosphoglucomutase and glucose-6-phosphate dehydrogenase. The rate of reduction of NADP during the reaction is stoichiometric with the rate of production of glucose-1-phosphate from the starch substrate. Reduction of NADP was followed at 340 nm in a Varian Cary double-beam spectrophotometer. Protein levels in extracts were determined according to Bradford (1976).

Glucan phosphorylase activity gels were run essentially according to Steup (1990). Proteins were separated on native polyacrylamide gels (8.5 %) containing 1.5 % glycogen. Following electrophoresis at 80 V for 15 h (4°C), the gels were incubated (1-2 h) at 37°C in 0.1 M citrate-NaOH buffer (pH 6.0) containing 20 mM glucose-1-P and 0.05% (w/v) hydrolyzed potato starch. Gels were then rinsed and stained with an iodine solution. For Western blot analysis, proteins were electrophoresed on glycogen-containing polyacrylamide gels as described above. The proteins were electroblotted to nitrocellulose and blots were probed with polyclonal antibodies raised against GHTP and GLTP.

1 Immunoblots were developed with alkaline phosphatase conjugated anti-rabbit secondary
2 antibodies (Sigma).

4 H Chip Color Determination

5 Five transgenic potato lines expressing the GLTP antisense sequence, two transgenic
6 lines expressing the GHTP antisense sequence, non-transgenic Desiree control lines, and two
7 control lines transformed with the pBI121 vector T-DNA, were grown under field conditions
8 in Alberta, Canada. Tubers were harvested and stored at 10°C and 4°C. Chip color was
9 determined for all potato lines by taking center cuts from representative samples from each
10 line and frying at 205°F in soybean oil for approximately 3 minutes until bubbling stops.

12 I Results

13 All tubers were harvested from plants of the same cultivar (Desiree), the same age,
14 and grown side by side under identical growth conditions. Northern analysis of tubers
15 showed a considerable reduction of endogenous GLTP transcript in transgenic plants
16 expressing the homologous antisense transcript (Figure 5). Glucan phosphorylase assays
17 showed that activities ($\mu\text{mol NADPH mg}^{-1} \text{ protein h}^{-1}$) were reduced (Table 1) at harvest and
18 for at least six months following harvest in transgenic plants expressing the GLTP antisense
19 DNA. The results tabulated in Table 1 show that α glucan phosphorylase activity in tubers
20 stored at 4°C for 189 days was reduced from approximately 16% to 70% in various
21 transformed potato varieties relative to the wildtype control strain. Activity gels and western
22 blot analysis showed specific reduced expression of homologous enzymes and lower
23 reduction of expression for heterologous enzymes (Figure 8). This specificity for
24 homologous products may result from differences between the phosphorylases (Figures 3 and
25 4).

26 Analysis of tubers at harvest (0 days) shows that those expressing the antisense GLTP
27 transcript have up to 5-fold less reducing sugars than control tubers (Table 2). Furthermore,
28 after 91 days storage at 4°C, transformed tubers contained 28-39% lower reducing sugar
29 concentrations than the wildtype control strain. Concentrations of glucose and fructose were
30 reduced significantly in tubers expressing the antisense GLTP transcript (Tables 3 and 4).
31 These results suggest that reduced GLTP activity slows the catabolism of starch into reducing

1 sugars in tubers, while in the control tubers the sugars continue to accumulate. The
2 correlation between total phosphorylase activity and the concentration of reducing sugars is
3 not direct, suggesting that certain isozymes of phosphorylase may play a more important role
4 in the catabolism of starch, that specific levels of reduced expression of particular
5 phosphorylase isozymes may be more optimum than others, and/or that there may be
6 unidentified interactions involved in the lower reducing sugar levels.

7 Transgenic potato plants expressing the antisense GLTP or GHTP transcript have
8 been grown under field conditions and their tubers stored at 4°C. Chip color, which
9 correlated with sugar content, was determined prior to cold storage and after 86 and 124 days
10 of cold storage. The chip color of tubers from all transgenic plants expressing the antisense
11 GLTP transcript was significantly improved (lighter) relative to that of control tubers (darker)
12 stored under identical conditions (Table 5 and Figure 7). Chip scores of tubers from
13 "Desiree" potato plants expressing the GLTP transcript were improved by at least 4.3 points
14 and 8.9 points as determined with an Agtron model E-15-FP Direct Reading Abridged
15 Spectrophotometer (Agtron Inc. 1095 Spice Island Drive #100, Sparks Nevada 89431)
16 following storage at 10°C and 4°C, respectively, for 86 days. Chip scores of GLTP
17 transformants measured after 124 days of storage at 4°C were improved by 44% to 89%
18 relative to wildtype (Table 5).

19 The Desiree cultivar is not a commercially desirable potato for chipping due to its
20 high natural sugar content and propensity to sweeten rapidly in cold storage. Nevertheless,
21 significant improvements in fried chip color were noted with the transformed "Desiree"
22 potatoes. It is expected that superior color lightening would be achieved if the methods of the
23 invention were applied to commercial processing potato varieties.

24 Analysis of tubers stored at 10°C and 4°C shows that those expressing the antisense
25 GHTP transcript sometimes provided chips that fried lighter than control tubers, indicating a
26 lower buildup of reducing sugars (Table 5). Results showing heterologous and homologous
27 reduction in phosphorylase activity (Figure 8) indicate that the improvement may be a result
28 of reducing one or both tuber phosphorylases. However, these results suggest that the L-type
29 phosphorylase plays a more important role in the catabolism of starch into reducing sugars.

30 Further, the results show that the difference in reducing sugar levels (Table 2) and
31 chip scores (Table 5) between tubers wildtype plants and those expressing tuber

1 phosphorylase antisense RNA, are sustained during long-term storage. As shown in Table 5,
2 the chip scores are approximately the same at 86 days and 124 days. No further increases in
3 reducing sugar concentrations were evident after 49 and 91 days storage at 4°C (Table 2).
4 This equilibrium in sugar concentration was probably associated with the kinetics of the tuber
5 phosphorylases. The capability of maintaining lower sugar levels has the potential of
6 extending the period of storage by at least several months. Presently, processing potatoes are
7 usually stored for a maximum of three to six months at 10°C to 12°C before the sugar
8 accumulation reaches levels that reduce quality. Fresh product must be imported until the
9 present season potatoes become available. Extending the storage period of potatoes by many
10 months may reduce import costs.

11 Table 6 provides a summary of the percentage improvement in various improved tuber
12 cold-storage characteristics of tubers of potato plants transformed with antisense DNA
13 derived from the GLTP gene sequence (ATL3 - ATL9), and from the GHTP gene sequence
14 (ATH1 and ATH2) relative to untransformed control plants. It is apparent from the results
15 summarized in Table 6 that substantial improvements in tuber cold-storage characteristics
16 may be consistently obtained through the methods of the present invention. Particularly
17 noteworthy are the percentage chip score improvements over wildtype observed after storage
18 at 4°C for approximately four months (124 days). Relative chip score improvements of up to
19 89% relative to wildtype were observed. Improved chip scores reflect the commercial utility
20 of the invention. That is, by reducing cold-induced sweetening, tubers can be stored at cooler
21 temperatures, without causing unacceptable darkening of fried potato products.

22 The reduction in sugar accumulation of transformed potato lines relative to wildtype,
23 both at harvest and after 91 day storage, also demonstrates significant advantages of the
24 invention. Reduced sugar accumulation relates to the observed chip score improvements, and
25 also reflects improved specific gravity of tubers, another important commercial measure of
26 tuber quality.

27 Even at harvest, substantial improvements in chip score and reduced sugar
28 accumulation were noted for transformed lines relative to wildtype. Thus, the benefits of the
29 invention are not limited to improvements that arise only after extended periods of cold
30 storage, but that are present at the time of harvest. In this sense, the invention is not limited
31 only to improvements in cold-storage characteristics but encompasses improvements in tuber

1 quality characteristics such as chip score or sugar accumulation which are present at the time
2 of harvest, resulting in earlier maturity.

3 Turning to specific improvements summarized in Table 6, it can be seen that GLTP-
4 type transformants (ATL3 - ATL9) exhibited up to a 66%, 70% and 69% reduction in α
5 glucan phosphorylase activity relative to wildtype, at harvest, and after storage for 91 and 189
6 days, respectively. Most also exhibited improvements in excess of 10% and 30% relative to
7 wildtype at harvest and after storage for 91 and 189 days. After storage for 91 and 189 days,
8 the GHTP-type transformants (ATH1 and ATH2) exhibited, respectively, up to 28% and 39%
9 relative improvement over wildtype and generally showed at least 10% improvement.

10 The GLTP-type transformants exhibited up to 80% and 39% reduction of sugar
11 accumulation relative to wildtype at harvest and at 91 days, respectively. At harvest, all
12 GLTP-type transformants exhibited at least 10% and at least 30% relative improvement. At
13 91 days, all GLTP-type transformants exhibited at least 10% and most exhibited at least 30%
14 relative improvement.

15 The GLTP-type transformants exhibited up to 46%, 89% and 89% chip score
16 improvement relative to wildtype at harvest, and after storage for 86 days and 124 days,
17 respectively. Almost all exhibited at least 10% and 30% relative improvement at harvest, and
18 after storage for 86 and 124 days. At least one of the GHTP-type transformants exhibited at
19 least 5% and at least 10% improvement relative to wildtype at harvest, and after storage for
20 86 and 124 days. After 124 days storage, at least one of the GHTP-type transformants
21 exhibited up to 25% relative improvement in chip score.

22 The results clearly demonstrate that substantial improvements in tuber cold-storage
23 characteristics may be readily obtained through the methods of the invention. Results will
24 vary due to, among other things, the location within the plant genome where the recombinant
25 antisense or sense DNA is inserted, and the number of insertion events that occur. It is
26 important to note that despite the variability in the results amongst the various transformed
27 lines, there was little variation in the results amongst the samples within a single transformed
28 potato line (see footnotes to Tables 1 to 5). Results are presented in Table 6 for all potato
29 plant lines which were successfully transformed with the GHTP or GLTP antisense DNA.
30 Therefore, all transformants show at least some improvement in one or more cold-storage
31 characteristics such as increased chip score (lighter color on cooking) and reduced sugar

1 accumulation, and most show very substantial improvements. Given the large proportion of
2 positive transformants observed in the examples herein, it is expected that, using the cold-
3 storage characteristic testing procedures described in the examples, potato plants transformed
4 through the methods of the invention can be readily screened to identify transformed lines
5 exhibiting significantly improved cold-storage characteristics. By applying the techniques
6 disclosed herein to commercially important potato varieties, it will be possible to readily
7 create and select transformants having significantly improved cold-storage characteristics.
8 Those transformants showing the greatest relative improvements over wildtype controls can
9 be used in the development of new commercial potato varieties.

10

Table 1

Effects of an antisense transcript on glucan phosphorylase activity measured in enzyme extracts from field grown "Desiree" tubers.

Glucan Phosphorylase Activity Storage Period at 4C (days)					
Clone	0	49	91	140	189
	$\mu\text{mol NADPH mg}^{-1} \text{ protein h}^{-1}$				
Wt ^a	10.50	11.83	9.94	11.90	13.04
ATL3	4.90	4.86	4.49	4.73	4.88
ATL4	11.45	7.17	8.09	11.32	10.99
ATL5	3.58	3.56	2.97	4.59	4.79
ATL9	3.59	3.88	3.84	4.72	3.98
LSD _{0.05} ^b	1.97	2.94	1.59	2.34	2.58
LSD _{0.01}	2.87	4.28	2.31	3.41	3.75
Clone ^c		0.01 ^d			
WT vs. ATL's		0.01			
Days		NS			
Clone x Days		0.05			
WT		11.49	8.90	12.66	13.66
ATH-1		10.40	9.69	10.79	10.10
ATH-2		6.46	6.40	6.56	8.38
LSD _{0.05} ^b		2.02	0.41	3.00	NS
LSD _{0.01}		4.78	0.95	NS	NS
Clone ^c		0.01			
WT vs. ATH's		0.01			
Days		0.05			
Clone x Days		NS			

^aWT, wild type untransformed tubers. ^bLSD, least significant difference at 0.05 or 0.01 level for each storage period. ^cSources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

Table 2

Effects of an antisense GLTP transcript on low temperature induced sweetening of field grown "Desiree" tubers.

Clone	Reducing Sugars (glucose + fructose) Storage Period at 4C (days)		
	0	49	91
	mg g ⁻¹ fresh weight		
Wt ^a	5.63	31.8	28.0
ATL3	1.88	17.3	17.3
ATL4	1.11	14.3	20.1
ATL5	1.51	18.3	17.0
ATL9	1.36	17.3	18.5
WT vs. ATL's ^b	0.01	0.01	0.05
Clone ^c		0.01 ^d	
Days		0.01	
Clone x Days		NS	

^aWT, wild type untransformed tubers. ^bOrthogonal comparisons for ANOVA's at each storage period. ^csources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

Table 3

Effects of an antisense GLTP transcript on low temperature induced fructose accumulation of field grown "Desiree" tubers.

Clone	Fructose Storage Period at 4C (days)		
	0	49	91
	mg g ⁻¹ fresh weight		
Wt ^a	3.53	15.10	12.20
ATL3	1.21	8.40	8.79
ATL4	0.79	7.22	8.56
ATL5	0.61	10.00	8.09
ATL9	0.54	8.38	8.72
WT vs. ATL's ^b	0.01	0.01	NS
Clone ^c		0.01 ^d	
Days		0.01	
Clone x Days		NS	

^aWT, wild type untransformed tubers. ^bOrthogonal comparisons for ANOVA's at each storage period. ^cSources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

Table 4

Effects of an antisense GLTP transcript on low temperature induced glucose accumulation of field grown "Desiree" tubers.

Clone	Glucose Storage Period at 4C (days)		
	0	49	91
	mg g ⁻¹ fresh weight		
Wt ^a	2.10	16.60	15.90
ATL3	0.68	8.94	8.49
ATL4	0.32	7.07	11.06
ATL5	1.05	8.33	8.91
ATL9	0.83	8.87	9.78
WT vs. ATL's ^b	0.01	0.01	0.05
Clone ^c		0.01 ^d	
Days		0.01	
Clone x Days		NS	

^aWT, wild type untransformed tubers. ^bOrthogonal comparisons for ANOVA's at each storage period. ^csources of variation in factorial analysis. ^dSignificance levels for indicated sources of variation.

Table 5

Average chip color of field grown "Desiree" tubers. The chip color rating was assigned using an Agtron meter similar to that used by industry to measure color of fried potatoes. In this index, the higher the number the lighter the chip product but color does not represent a linear relationship to the index.

	Storage Temperature, Period, and Agtron Reading ^a			
	Harvest	86 days	86 days	124 days
		10C	4C	4C
Wt ^b	26	25.3	15.4	17.1
ATL3 ^c	25	37.4	26.7	30.8
ATL4	35	43.7	29.1	32.3
ATL5	36	29.6	24.7	24.6
ATL9	38	38.7	24.3	26.6
ATH1 ^d	26	49.7	17.5	21.4
ATH2	29	31.2	15.6	15.9
GMP1 ^e	31		15.7	15.7
GMP2	35		16.7	16.6

^aAgtron Inc. 1095 Spice Island Drive #100, Sparks Nevada 89431. Agtron model E-15-FP (Direct Reading Abridged Spectrophotometer). Measures ratio of reflectance in two spectral modes, near infrared and green. Results represent the measurement of 6 to 8 chips from 3 randomly selected tubers approximately 3 to 4 cm in diameter.

^bWT, negative control, wild type untransformed tubers.

^cATL, tubers transformed with the tuber L-type α glucan phosphorylase.

^dATH, tubers transformed with the tuber H-type α glucan phosphorylase.

^eGMP, negative control, tubers transformed with pBI121 T-DNA.

Table 6

Summary of Results

Sample	% Reduction of α glucan phosphorylase activity relative to wildtype			% Reduction of Sugar Accumulation relative to wildtype		% Chip Score Improvement relative to wildtype		
	at harvest	91 days	189 days	at harvest	91 days	at harvest	86 days	124 days
ATL 3	53	55	63	67	38	-4	73	80
ATL 4	-9	19	16	80	28	35	89	89
ATL 5	66	70	63	73	39	38	60	44
ATL 9	66	61	69	76	34	46	58	56
ATH 1	n/a	-9	26	n/a	n/a	0	14	25
ATH 2	n/a	28	39	n/a	n/a	12	1	-7

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14
15 All publications mentioned in this specification are indicative of the level of skill in
16 the art to which this invention pertains. All publications are herein incorporated by reference
17 to the same extent as if each individual publication was specifically and individually indicated
18 to be incorporated by reference.

19 Although the foregoing invention has been described in some detail by way of
20 illustration and example for purposes of clarity of understanding, it will be obvious that
21 certain changes and modifications may be practised within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Her Majesty the Queen in Right of Canada as Represented
by the Department of Agriculture and Agri-Food Canada
- (ii) TITLE OF INVENTION: Potatoes Having Improved Quality
Characteristics and Methods for Their Production
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: McKay-Carey & Company
 - (B) STREET: 2125 Commerce Place, 10155-102nd Street
 - (C) CITY: Edmonton
 - (D) STATE: Alberta
 - (E) COUNTRY: Canada
 - (F) ZIP: T5J 4G8
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: WO
 - (B) FILING DATE: 10-FEB-1998
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/036,946
 - (B) FILING DATE: 10-FEB-1997
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/868,786
 - (B) FILING DATE: 04-JUN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: McKay-Carey, Mary Jane
 - (B) REGISTRATION NUMBER: 3790
 - (C) REFERENCE/DOCKET NUMBER: 24002W00
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (403) 424-0222
 - (B) TELEFAX: (403) 421-0834

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3101 base pairs
 - (B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Solanum tuberosum*

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 44..2944

(D) OTHER INFORMATION: /product= "potato alpha-glucan
L-type tuber phosphorylase"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide

(B) LOCATION: 194..2941

(ix) FEATURE:

(A) NAME/KEY: sig_peptide

(B) LOCATION: 44..193.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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						-50										
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Asn	Gly	Ala	His	Leu	Phe	Asn	His	Tyr	Ser	Ser	Asn	Ser	Arg	Phe	Ile	
	-45					-40					-35					
CAT	TTC	ACT	TCT	AGA	AAC	ACA	AGC	TCC	AAA	TTG	TTC	CTT	ACC	AAA	ACC	151
His	Phe	Thr	Ser	Arg	Asn	Thr	Ser	Ser	Lys	Leu	Phe	Leu	Thr	Lys	Thr	
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TCC	CAT	TTT	CGG	AGA	CCC	AAA	CGC	TGT	TTC	CAT	GTC	AAC	AAT	ACC	TTG	199
Ser	His	Phe	Arg	Arg	Pro	Lys	Arg	Cys	Phe	His	Val	Asn	Asn	Thr	Leu	
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AGT	GAG	AAA	ATT	CAC	CAT	CCC	ATT	ACT	GAA	CAA	GGT	GGT	GAG	AGC	GAC	247
Ser	Glu	Lys	Ile	His	His	Pro	Ile	Thr	Glu	Gln	Gly	Gly	Glu	Ser	Asp	
	5					10					15					
CTG	AGT	TCT	TTT	GCT	CCT	GAT	GCC	GCA	TCT	ATT	ACC	TCA	AGT	ATC	AAA	295
Leu	Ser	Ser	Phe	Ala	Pro	Asp	Ala	Ala	Ser	Ile	Thr	Ser	Ser	Ile	Lys	
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TAC	CAT	GCA	GAA	TTC	ACA	CCT	GTA	TTC	TCT	CCT	GAA	AGG	TTT	GAG	CTC	343
Tyr	His	Ala	Glu	Phe	Thr	Pro	Val	Phe	Ser	Pro	Glu	Arg	Phe	Glu	Leu	
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CCT	AAG	GCA	TTC	TTT	GCA	ACA	GCT	CAA	AGT	GTT	CGT	GAT	TCG	CTC	CTT	391
Pro	Lys	Ala	Phe	Phe	Ala	Thr	Ala	Gln	Ser	Val	Arg	Asp	Ser	Leu	Leu	
				55					60					65		
ATT	AAT	TGG	AAT	GCT	ACG	TAT	GAT	ATT	TAT	GAA	AAG	CTG	AAC	ATG	AAG	439
Ile	Asn	Trp	Asn	Ala	Thr	Tyr	Asp	Ile	Tyr	Glu	Lys	Leu	Asn	Met	Lys	
			70					75					80			
CAA	GCG	TAC	TAT	CTA	TCC	ATG	GAA	TTT	CTG	CAG	GGT	AGA	GCA	TTG	TTA	487
Gln	Ala	Tyr	Tyr	Leu	Ser	Met	Glu	Phe	Leu	Gln	Gly	Arg	Ala	Leu	Leu	
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AAT	GCA	ATT	GGT	AAT	CTG	GAG	CTT	ACT	GGT	GCA	TTT	GCG	GAA	GCT	TTG	535
Asn	Ala	Ile	Gly	Asn	Leu	Glu	Leu	Thr	Gly	Ala	Phe	Ala	Glu	Ala	Leu	
100						105					110					
AAA	AAC	CTT	GGC	CAC	AAT	CTA	GAA	AAT	GTG	GCT	TCT	CAG	GAA	CCA	GAT	583
Lys	Asn	Leu	Gly	His	Asn	Leu	Glu	Asn	Val	Ala	Ser	Gln	Glu	Pro	Asp	
115					120					125					130	
GCT	GCT	CTT	GGA	AAT	GGG	GGT	TTG	GGA	CGG	CTT	GCT	TCC	TGT	TTT	CTG	631
Ala	Ala	Leu	Gly	Asn	Gly	Gly	Leu	Gly	Arg	Leu	Ala	Ser	Cys	Phe	Leu	
				135					140					145		
GAC	TCT	TTG	GCA	ACA	CTA	AAC	TAC	CCA	GCA	TGG	GGC	TAT	GGA	CTT	AGG	679
Asp	Ser	Leu	Ala	Thr	Leu	Asn	Tyr	Pro	Ala	Trp	Gly	Tyr	Gly	Leu	Arg	
			150					155					160			
TAC	AAG	TAT	GGT	TTA	TTT	AAG	CAA	CGG	ATT	ACA	AAA	GAT	GGT	CAG	GAG	727
Tyr	Lys	Tyr	Gly	Leu	Phe	Lys	Gln	Arg	Ile	Thr	Lys	Asp	Gly	Gln	Glu	
		165					170					175				
GAG	GTG	GCT	GAA	GAT	TGG	CTT	GAA	ATT	GGC	AGT	CCA	TGG	GAA	GTT	GTG	775
Glu	Val	Ala	Glu	Asp	Trp	Leu	Glu	Ile	Gly	Ser	Pro	Trp	Glu	Val	Val	
180						185					190					
AGG	AAT	GAT	GTT	TCA	TAT	CCT	ATC	AAA	TTC	TAT	GGA	AAA	GTC	TCT	ACA	823
Arg	Asn	Asp	Val	Ser	Tyr	Pro	Ile	Lys	Phe	Tyr	Gly	Lys	Val	Ser	Thr	
195					200					205					210	
GGA	TCA	GAT	GGA	AAG	AGG	TAT	TGG	ATT	GGT	GGA	GAG	GAT	ATA	AAG	GCA	871
Gly	Ser	Asp	Gly	Lys	Arg	Tyr	Trp	Ile	Gly	Gly	Glu	Asp	Ile	Lys	Ala	
				215					220				225			
GTT	GCG	TAT	GAT	GTT	CCC	ATA	CCA	GGG	TAT	AAG	ACC	AGA	ACC	ACA	ATC	919
Val	Ala	Tyr	Asp	Val	Pro	Ile	Pro	Gly	Tyr	Lys	Thr	Arg	Thr	Thr	Ile	
			230					235					240			
AGC	CTT	CGA	CTG	TGG	TCT	ACA	CAG	GTT	CCA	TCA	GCG	GAT	TTT	GAT	TTA	967
Ser	Leu	Arg	Leu	Trp	Ser	Thr	Gln	Val	Pro	Ser	Ala	Asp	Phe	Asp	Leu	
		245					250					255				

TCT GCT TTC AAT GCT GGA GAG CAC ACC AAA GCA TGT GAA GCC CAA GCA Ser Ala Phe Asn Ala Gly Glu His Thr Lys Ala Cys Glu Ala Gln Ala 260 265 270	1015
AAC GCT GAG AAG ATA TGT TAC ATA CTC TAC CCT GGG GAT GAA TCA GAG Asn Ala Glu Lys Ile Cys Tyr Ile Leu Tyr Pro Gly Asp Glu Ser Glu 275 280 285 290	1063
GAG GGA AAG ATC CTT CGG TTG AAG CAA CAA TAT ACC TTA TGC TCG GCT Glu Gly Lys Ile Leu Arg Leu Lys Gln Gln Tyr Thr Leu Cys Ser Ala 295 300 305	1111
TCT CTC CAA GAT ATT ATT TCT CGA TTT GAG AGG AGA TCA GGT GAT CGT Ser Leu Gln Asp Ile Ile Ser Arg Phe Glu Arg Arg Ser Gly Asp Arg 310 315 320	1159
ATT AAG TGG GAA GAG TTT CCT GAA AAA GTT GCT GTG CAG ATG AAT GAC Ile Lys Trp Glu Glu Phe Pro Glu Lys Val Ala Val Gln Met Asn Asp 325 330 335	1207
ACT CAC CCT ACA CTT TGT ATC CCT GAG CTG ATG AGA ATA TTG ATA GAT Thr His Pro Thr Leu Cys Ile Pro Glu Leu Met Arg Ile Leu Ile Asp 340 345 350	1255
CTG AAG GGC TTG AAT TGG AAT GAA GCT TGG AAT ATT ACT CAA AGA ACT Leu Lys Gly Leu Asn Trp Asn Glu Ala Trp Asn Ile Thr Gln Arg Thr 355 360 365 370	1303
GTG GCC TAC ACA AAC CAT ACT GTT TTG CCT GAG GCA CTG GAG AAA TGG Val Ala Tyr Thr Asn His Thr Val Leu Pro Glu Ala Leu Glu Lys Trp 375 380 385	1351
AGT TAT GAA TTG ATG CAG AAA CTC CTT CCC AGA CAT GTC GAA ATC ATT Ser Tyr Glu Leu Met Gln Lys Leu Leu Pro Arg His Val Glu Ile Ile 390 395 400	1399
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CCT GAA ATC TCA GTT GAT GAT GAT ACT GAA ACA GTA GAA GTC CAT GAC Pro Glu Ile Ser Val Asp Asp Asp Thr Glu Thr Val Glu Val His Asp 455 460 465	1591
AAA GTT GAA GCT TCC GAT AAA GTT GTG ACT AAT GAT GAA GAT GAC ACT Lys Val Glu Ala Ser Asp Lys Val Val Thr Asn Asp Glu Asp Asp Thr 470 475 480	1639

GGT AAG AAA ACT AGT GTG AAG ATA GAA GCA GCT GCA GAA AAA GAC ATT	1687
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Asp Lys Lys Thr Pro Val Ser Pro Glu Pro Ala Val Ile Pro Pro Lys	
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Asp Phe Tyr Glu Leu Trp Pro Glu Lys Phe Gln Asn Lys Thr Asn Gly	
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GTG ACT CCA AGA AGA TGG ATT CGT TTC TGC AAT CCT CCT CTT AGT GCC	1927
Val Thr Pro Arg Arg Trp Ile Arg Phe Cys Asn Pro Pro Leu Ser Ala	
565 570 575	
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580 585 590	
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GAG TGG AGG GAA GCA AAA AGG AGC AAC AAG ATT AAA GTT GTC TCC TTT	2071
Glu Trp Arg Glu Ala Lys Arg Ser Asn Lys Ile Lys Val Val Ser Phe	
615 620 625	
CTC AAA GAA AAG ACA GGG TAT TCT GTT GTC CCA GAT GCA ATG TTT GAT	2119
Leu Lys Glu Lys Thr Gly Tyr Ser Val Val Pro Asp Ala Met Phe Asp	
630 635 640	
ATT CAG GTA AAA CGC ATT CAT GAG TAC AAG CGA CAA CTG TTA AAT ATC	2167
Ile Gln Val Lys Arg Ile His Glu Tyr Lys Arg Gln Leu Leu Asn Ile	
645 650 655	
TTC GGC ATC GTT TAT CGG TAT AAG AAG ATG AAA GAA ATG ACA GCT GCA	2215
Phe Gly Ile Val Tyr Arg Tyr Lys Lys Met Lys Glu Met Thr Ala Ala	
660 665 670	
GAA AGA AAG ACT AAC TTC GTT CCT CGA GTA TGC ATA TTT GGG GGA AAA	2263
Glu Arg Lys Thr Asn Phe Val Pro Arg Val Cys Ile Phe Gly Gly Lys	
675 680 685 690	
GCT TTT GCC ACA TAT GTG CAA GCC AAG AGG ATT GTA AAA TTT ATC ACA	2311
Ala Phe Ala Thr Tyr Val Gln Ala Lys Arg Ile Val Lys Phe Ile Thr	
695 700 705	

GAT GTT GGT GCT ACT ATA AAT CAT GAT CCA GAA ATC GGT GAT CTG TTG Asp Val Gly Ala Thr Ile Asn His Asp Pro Glu Ile Gly Asp Leu Leu 710 715 720	2359
AAG GTA GTC TTT GTG CCA GAT TAC AAT GTC AGT GTT GCT GAA TTG CTA Lys Val Val Phe Val Pro Asp Tyr Asn Val Ser Val Ala Glu Leu Leu 725 730 735	2407
ATT CCT GCT AGC GAT CTA TCA GAA CAT ATC AGT ACG GCT GGA ATG GAG Ile Pro Ala Ser Asp Leu Ser Glu His Ile Ser Thr Ala Gly Met Glu 740 745 750	2455
GCC AGT GGA ACC AGT AAT ATG AAG TTT GCA ATG AAT GGT TGT ATC CAA Ala Ser Gly Thr Ser Asn Met Lys Phe Ala Met Asn Gly Cys Ile Gln 755 760 765 770	2503
ATT GGT ACA TTG GAT GGC GCT AAT GTT GAA ATA AGG GAA GAG GTT GGA Ile Gly Thr Leu Asp Gly Ala Asn Val Glu Ile Arg Glu Glu Val Gly 775 780 785	2551
GAA GAA AAC TTC TTT CTC TTT GGT GCT CAA GCT CAT GAA ATT GCA GGG Glu Glu Asn Phe Phe Leu Phe Gly Ala Gln Ala His Glu Ile Ala Gly 790 795 800	2599
CTT AGA AAA GAA AGA GCT GAC GGA AAG TTT GTA CCT GAT GAA CGT TTT Leu Arg Lys Glu Arg Ala Asp Gly Lys Phe Val Pro Asp Glu Arg Phe 805 810 815	2647
GAA GAG GTG AAG GAA TTT GTT AGA AGC GGT GCT TTT GGC TCT TAT AAC Glu Glu Val Lys Glu Phe Val Arg Ser Gly Ala Phe Gly Ser Tyr Asn 820 825 830	2695
TAT GAT GAC CTA ATT GGA TCG TTG GAA GGA AAT GAA GGT TTT GGC CGT Tyr Asp Asp Leu Ile Gly Ser Leu Glu Gly Asn Glu Gly Phe Gly Arg 835 840 845 850	2743
GCT GAC TAT TTC CTT GTG GGC AAG GAC TTC CCC AGT TAC ATA GAA TGC Ala Asp Tyr Phe Leu Val Gly Lys Asp Phe Pro Ser Tyr Ile Glu Cys 855 860 865	2791
CAA GAG AAA GTT GAT GAG GCA TAT CGC GAC CAG AAA AGG TGG ACA ACG Gln Glu Lys Val Asp Glu Ala Tyr Arg Asp Gln Lys Arg Trp Thr Thr 870 875 880	2839
ATG TCA ATC TTG AAT ACA GCG GGA TCG TAC AAG TTC AGC AGT GAC AGA Met Ser Ile Leu Asn Thr Ala Gly Ser Tyr Lys Phe Ser Ser Asp Arg 885 890 895	2887
ACA ATC CAT GAA TAT GCC AAA GAC ATT TGG AAC ATT GAA GCT GTG GAA Thr Ile His Glu Tyr Ala Lys Asp Ile Trp Asn Ile Glu Ala Val Glu 900 905 910	2935
ATA GCA TAA GAGGGGAAG TGAATGAAAA ATAACAAAGG CACAGTAAGT Ile Ala * 915	2984

AGTTTCTCTT TTTATCATGT GATGAAGGTA TATAATGTAT GTGTAAGAGG ATGATGTTAT 3044
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 967 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Ser	Arg	Phe	Ile	His	Phe	Thr	Ser	Arg	Asn	Thr	Ser	Ser	Lys	Leu	Phe	
			-30						-25					-20		
Leu	Thr	Lys	Thr	Ser	His	Phe	Arg	Arg	Pro	Lys	Arg	Cys	Phe	His	Val	
		-15					-10						-5			
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		1				5					10					
Gly	Glu	Ser	Asp	Leu	Ser	Ser	Phe	Ala	Pro	Asp	Ala	Ala	Ser	Ile	Thr	
15				20						25				30		
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			35						40					45		
Arg	Phe	Glu	Leu	Pro	Lys	Ala	Phe	Phe	Ala	Thr	Ala	Gln	Ser	Val	Arg	
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		65				70						75				
Leu	Asn	Met	Lys	Gln	Ala	Tyr	Tyr	Leu	Ser	Met	Glu	Phe	Leu	Gln	Gly	
	80					85					90					
Arg	Ala	Leu	Leu	Asn	Ala	Ile	Gly	Asn	Leu	Glu	Leu	Thr	Gly	Ala	Phe	
95				100						105				110		
Ala	Glu	Ala	Leu	Lys	Asn	Leu	Gly	His	Asn	Leu	Glu	Asn	Val	Ala	Ser	
			115						120					125		
Gln	Glu	Pro	Asp	Ala	Ala	Leu	Gly	Asn	Gly	Gly	Leu	Gly	Arg	Leu	Ala	
			130					135					140			
Ser	Cys	Phe	Leu	Asp	Ser	Leu	Ala	Thr	Leu	Asn	Tyr	Pro	Ala	Trp	Gly	
		145					150					155				

Tyr Gly Leu Arg Tyr Lys Tyr Gly Leu Phe Lys Gln Arg Ile Thr Lys
 160 165 170
 Asp Gly Gln Glu Glu Val Ala Glu Asp Trp Leu Glu Ile Gly Ser Pro
 175 180 185 190
 Trp Glu Val Val Arg Asn Asp Val Ser Tyr Pro Ile Lys Phe Tyr Gly
 195 200 205
 Lys Val Ser Thr Gly Ser Asp Gly Lys Arg Tyr Trp Ile Gly Gly Glu
 210 215 220
 Asp Ile Lys Ala Val Ala Tyr Asp Val Pro Ile Pro Gly Tyr Lys Thr
 225 230 235
 Arg Thr Thr Ile Ser Leu Arg Leu Trp Ser Thr Gln Val Pro Ser Ala
 240 245 250
 Asp Phe Asp Leu Ser Ala Phe Asn Ala Gly Glu His Thr Lys Ala Cys
 255 260 265 270
 Glu Ala Gln Ala Asn Ala Glu Lys Ile Cys Tyr Ile Leu Tyr Pro Gly
 275 280 285
 Asp Glu Ser Glu Glu Gly Lys Ile Leu Arg Leu Lys Gln Gln Tyr Thr
 290 295 300
 Leu Cys Ser Ala Ser Leu Gln Asp Ile Ile Ser Arg Phe Glu Arg Arg
 305 310 315
 Ser Gly Asp Arg Ile Lys Trp Glu Glu Phe Pro Glu Lys Val Ala Val
 320 325 330
 Gln Met Asn Asp Thr His Pro Thr Leu Cys Ile Pro Glu Leu Met Arg
 335 340 345 350
 Ile Leu Ile Asp Leu Lys Gly Leu Asn Trp Asn Glu Ala Trp Asn Ile
 355 360 365
 Thr Gln Arg Thr Val Ala Tyr Thr Asn His Thr Val Leu Pro Glu Ala
 370 375 380
 Leu Glu Lys Trp Ser Tyr Glu Leu Met Gln Lys Leu Leu Pro Arg His
 385 390 395
 Val Glu Ile Ile Glu Ala Ile Asp Glu Glu Leu Val His Glu Ile Val
 400 405 410
 Leu Lys Tyr Gly Ser Met Asp Leu Asn Lys Leu Glu Glu Lys Leu Thr
 415 420 425 430
 Thr Met Arg Ile Leu Glu Asn Phe Asp Leu Pro Ser Ser Val Ala Glu
 435 440 445

Leu Phe Ile Lys Pro Glu Ile Ser Val Asp Asp Asp Thr Glu Thr Val
 450 455 460
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 465 470 475
 Glu Asp Asp Thr Gly Lys Lys Thr Ser Val Lys Ile Glu Ala Ala Ala
 480 485 490
 Glu Lys Asp Ile Asp Lys Lys Thr Pro Val Ser Pro Glu Pro Ala Val
 495 500 505 510
 Ile Pro Pro Lys Lys Val Arg Met Ala Asn Leu Cys Val Val Gly Gly
 515 520 525
 His Ala Val Asn Gly Val Ala Glu Ile His Ser Glu Ile Val Lys Glu
 530 535 540
 Glu Val Phe Asn Asp Phe Tyr Glu Leu Trp Pro Glu Lys Phe Gln Asn
 545 550 555
 Lys Thr Asn Gly Val Thr Pro Arg Arg Trp Ile Arg Phe Cys Asn Pro
 560 565 570
 Pro Leu Ser Ala Ile Ile Thr Lys Trp Thr Gly Thr Glu Asp Trp Val
 575 580 585 590
 Leu Lys Thr Glu Lys Leu Ala Glu Leu Gln Lys Phe Ala Asp Asn Glu
 595 600 605
 Asp Leu Gln Asn Glu Trp Arg Glu Ala Lys Arg Ser Asn Lys Ile Lys
 610 615 620
 Val Val Ser Phe Leu Lys Glu Lys Thr Gly Tyr Ser Val Val Pro Asp
 625 630 635
 Ala Met Phe Asp Ile Gln Val Lys Arg Ile His Glu Tyr Lys Arg Gln
 640 645 650
 Leu Leu Asn Ile Phe Gly Ile Val Tyr Arg Tyr Lys Lys Met Lys Glu
 655 660 665 670
 Met Thr Ala Ala Glu Arg Lys Thr Asn Phe Val Pro Arg Val Cys Ile
 675 680 685
 Phe Gly Gly Lys Ala Phe Ala Thr Tyr Val Gln Ala Lys Arg Ile Val
 690 695 700
 Lys Phe Ile Thr Asp Val Gly Ala Thr Ile Asn His Asp Pro Glu Ile
 705 710 715
 Gly Asp Leu Leu Lys Val Val Phe Val Pro Asp Tyr Asn Val Ser Val
 720 725 730

Ala Glu Leu Leu Ile Pro Ala Ser Asp Leu Ser Glu His Ile Ser Thr
 735 740 745 750
 Ala Gly Met Glu Ala Ser Gly Thr Ser Asn Met Lys Phe Ala Met Asn
 755 760 765
 Gly Cys Ile Gln Ile Gly Thr Leu Asp Gly Ala Asn Val Glu Ile Arg
 770 775 780
 Glu Glu Val Gly Glu Glu Asn Phe Phe Leu Phe Gly Ala Gln Ala His
 785 790 795
 Glu Ile Ala Gly Leu Arg Lys Glu Arg Ala Asp Gly Lys Phe Val Pro
 800 805 810
 Asp Glu Arg Phe Glu Glu Val Lys Glu Phe Val Arg Ser Gly Ala Phe
 815 820 825 830
 Gly Ser Tyr Asn Tyr Asp Asp Leu Ile Gly Ser Leu Glu Gly Asn Glu
 835 840 845
 Gly Phe Gly Arg Ala Asp Tyr Phe Leu Val Gly Lys Asp Phe Pro Ser
 850 855 860
 Tyr Ile Glu Cys Gln Glu Lys Val Asp Glu Ala Tyr Arg Asp Gln Lys
 865 870 875
 Arg Trp Thr Thr Met Ser Ile Leu Asn Thr Ala Gly Ser Tyr Lys Phe
 880 885 890
 Ser Ser Asp Arg Thr Ile His Glu Tyr Ala Lys Asp Ile Trp Asn Ile
 895 900 905 910
 Glu Ala Val Glu Ile Ala *
 915

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2655 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

- (A) NAME/KEY: CDS

(B) LOCATION: 12..2528
 (D) OTHER INFORMATION: /product= "potato alpha-glucan
 H-type tuber phosphorylase"

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
 (B) LOCATION: 12..2525

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTTATTTTC C ATG GAA GGT GGT GCA AAA TCG AAT GAT GTA TCA GCA GCA	50
Met Glu Gly Gly Ala Lys Ser Asn Asp Val Ser Ala Ala	
1 5 10	
CCT ATT GCT CAA CCA CTT TCT GAA GAC CCT ACT GAC ATT GCA TCT AAT	98
Pro Ile Ala Gln Pro Leu Ser Glu Asp Pro Thr Asp Ile Ala Ser Asn	
15 20 25	
ATC AAG TAT CAT GCT CAA TAT ACT CCT CAT TTT TCT CCT TTC AAG TTT	146
Ile Lys Tyr His Ala Gln Tyr Thr Pro His Phe Ser Pro Phe Lys Phe	
30 35 40 45	
GAG CCA CTA CAA GCA TAC TAT GCT GCT ACT GCT GAC AGT GTT CGT GAT	194
Glu Pro Leu Gln Ala Tyr Tyr Ala Ala Thr Ala Asp Ser Val Arg Asp	
50 55 60	
CGC TTG ATC AAA CAA TGG AAT GAC ACC TAT CTT CAT TAT GAC AAA GTT	242
Arg Leu Ile Lys Gln Trp Asn Asp Thr Tyr Leu His Tyr Asp Lys Val	
65 70 75	
AAT CCA AAG CAA ACA TAC TAC TTA TCA ATG GAG TAT CTC CAG GGG CGA	290
Asn Pro Lys Gln Thr Tyr Tyr Leu Ser Met Glu Tyr Leu Gln Gly Arg	
80 85 90	
GCT TTG ACA AAT GCA GTT GGA AAC TTA GAC ATC CAC AAT GCA TAT GCT	338
Ala Leu Thr Asn Ala Val Gly Asn Leu Asp Ile His Asn Ala Tyr Ala	
95 100 105	
GAT GCT TTA AAC AAA CTG GGT CAG CAG CTT GAG GAG GTC GTT GAG CAG	386
Asp Ala Leu Asn Lys Leu Gly Gln Gln Leu Glu Glu Val Val Glu Gln	
110 115 120 125	
GAA AAA GAT GCA GCA TTA GGA AAT GGT GGT TTA GGA AGG CTC GCT TCA	434
Glu Lys Asp Ala Ala Leu Gly Asn Gly Gly Leu Gly Arg Leu Ala Ser	
130 135 140	
TGC TTT CTT GAT TCC ATG GCC ACA TTG AAC CTT CCA GCA TGG GGT TAT	482
Cys Phe Leu Asp Ser Met Ala Thr Leu Asn Leu Pro Ala Trp Gly Tyr	
145 150 155	
GGC TTG AGG TAC AGA TAT GGA CTT TTT AAG CAG CTT ATC ACA AAG GCT	530
Gly Leu Arg Tyr Arg Tyr Gly Leu Phe Lys Gln Leu Ile Thr Lys Ala	
160 165 170	

GGG CAA GAA GAA GTT CCT GAA GAT TGG TTG GAG AAA TTT AGT CCC TGG Gly Gln Glu Glu Val Pro Glu Asp Trp Leu Glu Lys Phe Ser Pro Trp 175 180 185	578
GAA ATT GTA AGG CAT GAT GTT GTC TTT CCT ATC AGG TTT TTT GGT CAT Glu Ile Val Arg His Asp Val Val Phe Pro Ile Arg Phe Phe Gly His 190 195 200 205	626
GTT GAA GTC CTC CCT TCT GGC TCG CGA AAA TGG GTT GGT GGA GAG GTC Val Glu Val Leu Pro Ser Gly Ser Arg Lys Trp Val Gly Gly Glu Val 210 215 220	674
CTA CAG GCT CTT GCA TAT GAT GTG CCA ATT CCA GGA TAC AGA ACT AAA Leu Gln Ala Leu Ala Tyr Asp Val Pro Ile Pro Gly Tyr Arg Thr Lys 225 230 235	722
AAC ACT AAT AGT CTT CGT CTC TGG GAA GCC AAA GCA AGC TCT GAG GAT Asn Thr Asn Ser Leu Arg Leu Trp Glu Ala Lys Ala Ser Ser Glu Asp 240 245 250	770
TTC AAC TTG TTT CTG TTT AAT GAT GGA CAG TAT GAT GCT GCT GCA CAG Phe Asn Leu Phe Leu Phe Asn Asp Gly Gln Tyr Asp Ala Ala Ala Gln 255 260 265	818
CTT CAT TCT AGG GCT CAG CAG ATT TGT GCT GTT CTC TAC CCT GGG GAT Leu His Ser Arg Ala Gln Gln Ile Cys Ala Val Leu Tyr Pro Gly Asp 270 275 280 285	866
GCT ACA GAG AAT GGA AAA CTC TTA CGG CTA AAG CAA CAA TTT TTT CTG Ala Thr Glu Asn Gly Lys Leu Leu Arg Leu Lys Gln Gln Phe Phe Leu 290 295 300	914
TGC AGT GCA TCG CTT CAG GAT ATT ATT GCC AGA TTC AAA GAG AGA GAA Cys Ser Ala Ser Leu Gln Asp Ile Ile Ala Arg Phe Lys Glu Arg Glu 305 310 315	962
GAT GGA AAG GGT TCT CAC CAG TGG TCT GAA TTC CCC AAG AAG GTT GCG Asp Gly Lys Gly Ser His Gln Trp Ser Glu Phe Pro Lys Lys Val Ala 320 325 330	1010
ATA CAA CTA AAT GAC ACA CAT CCA ACT CTT ACG ATT CCA GAG CTG ATG Ile Gln Leu Asn Asp Thr His Pro Thr Leu Thr Ile Pro Glu Leu Met 335 340 345	1058
CGG TTG CTA ATG GAT GAT GAA GGA CTT GGG TGG GAT GAA TCT TGG AAT Arg Leu Leu Met Asp Asp Glu Gly Leu Gly Trp Asp Glu Ser Trp Asn 350 355 360 365	1106
ATC ACT ACT AGG ACA ATT GCC TAT ACG AAT CAT ACA GTC CTA CCT GAA Ile Thr Thr Arg Thr Ile Ala Tyr Thr Asn His Thr Val Leu Pro Glu 370 375 380	1154
GCA CTT GAA AAA TGG TCT CAG GCA GTC ATG TGG AAG CTC CTT CCT AGA Ala Leu Glu Lys Trp Ser Gln Ala Val Met Trp Lys Leu Leu Pro Arg 385 390 395	1202

CAT ATG GAA ATC ATT GAA GAA ATT GAC AAA CGG TTT GTT GCT ACA ATA His Met Glu Ile Ile Glu Glu Ile Asp Lys Arg Phe Val Ala Thr Ile 400 405 410	1250
ATG TCA GAA AGA CCT GAT CTT GAG AAT AAG ATG CCT AGC ATG CGC ATT Met Ser Glu Arg Pro Asp Leu Glu Asn Lys Met Pro Ser Met Arg Ile 415 420 425	1298
TTG GAT CAC AAC GCC ACA AAA CCT GTT GTG CAT ATG GCT AAC TTG TGT Leu Asp His Asn Ala Thr Lys Pro Val Val His Met Ala Asn Leu Cys 430 435 440 445	1346
GTT GTC TCT TCA CAT ACG GTA AAT GGT GTT GCC CAG CTG CAT AGT GAC Val Val Ser Ser His Thr Val Asn Gly Val Ala Gln Leu His Ser Asp 450 455 460	1394
ATC CTG AAG GCT GAG TTA TTT GCT GAT TAT GTC TCT GTA TGG CCC ACC Ile Leu Lys Ala Glu Leu Phe Ala Asp Tyr Val Ser Val Trp Pro Thr 465 470 475	1442
AAG TTC CAG AAT AAG ACC AAT GGT ATA ACT CCT CGT AGG TGG ATC CGA Lys Phe Gln Asn Lys Thr Asn Gly Ile Thr Pro Arg Arg Trp Ile Arg 480 485 490	1490
TTT TGT AGT CCT GAG CTG AGT CAT ATA ATT ACC AAG TGG TTA AAA ACA Phe Cys Ser Pro Glu Leu Ser His Ile Ile Thr Lys Trp Leu Lys Thr 495 500 505	1538
GAT CAA TGG GTG ACG AAC CTC GAA CTG CTT GCT AAT CTT CGG GAG TTT Asp Gln Trp Val Thr Asn Leu Glu Leu Leu Ala Asn Leu Arg Glu Phe 510 515 520 525	1586
GCT GAT AAT TCG GAG CTC CAT GCT GAA TGG GAA TCA GCC AAG ATG GCC Ala Asp Asn Ser Glu Leu His Ala Glu Trp Glu Ser Ala Lys Met Ala 530 535 540	1634
AAC AAG CAG CGT TTG GCA CAG TAT ATA CTG CAT GTG ACA GGT GTG AGC Asn Lys Gln Arg Leu Ala Gln Tyr Ile Leu His Val Thr Gly Val Ser 545 550 555	1682
ATC GAT CCA AAT TCC CTT TTT GAC ATA CAA GTC AAA CGT ATC CAT GAA Ile Asp Pro Asn Ser Leu Phe Asp Ile Gln Val Lys Arg Ile His Glu 560 565 570	1730
TAC AAA AGG CAG CTT CTA AAT ATT CTG GGC GTC ATC TAT AGA TAC AAG Tyr Lys Arg Gln Leu Leu Asn Ile Leu Gly Val Ile Tyr Arg Tyr Lys 575 580 585	1778
AAG CTT AAG GGA ATG AGC CCT GAA GAA AGG AAA AAT ACA ACT CCT CGC Lys Leu Lys Gly Met Ser Pro Glu Glu Arg Lys Asn Thr Thr Pro Arg 590 595 600 605	1826
ACA GTC ATG ATT GGA GGA AAA GCA TTT GCA ACA TAC ACA AAT GCA AAA Thr Val Met Ile Gly Gly Lys Ala Phe Ala Thr Tyr Thr Asn Ala Lys 610 615 620	1874

CGA	ATT	GTC	AAG	CTC	GTG	ACT	GAT	GTT	GGC	GAC	GTT	GTC	AAT	AGT	GAC	1922
Arg	Ile	Val	Lys	Leu	Val	Thr	Asp	Val	Gly	Asp	Val	Val	Asn	Ser	Asp	
			625						630						635	
CCT	GAC	GTC	AAT	GAC	TAT	TTG	AAG	GTG	GTT	TTT	GTT	CCC	AAC	TAC	AAT	1970
Pro	Asp	Val	Asn	Asp	Tyr	Leu	Lys	Val	Val	Phe	Val	Pro	Asn	Tyr	Asn	
			640						645						650	
GTA	TCT	GTG	GCA	GAG	ATG	CTT	ATT	CCG	GGA	AGT	GAG	CTA	TCA	CAA	CAC	2018
Val	Ser	Val	Ala	Glu	Met	Leu	Ile	Pro	Gly	Ser	Glu	Leu	Ser	Gln	His	
			655						660						665	
ATC	AGT	ACT	GCA	GGC	ATG	GAA	GCA	AGT	GGA	ACA	AGC	AAC	ATG	AAA	TTT	2066
Ile	Ser	Thr	Ala	Gly	Met	Glu	Ala	Ser	Gly	Thr	Ser	Asn	Met	Lys	Phe	
					675										685	
GCC	CTT	AAT	GGA	TGC	CTT	ATC	ATT	GGG	ACA	CTA	GAT	GGG	GCC	AAT	GTG	2114
Ala	Leu	Asn	Gly	Cys	Leu	Ile	Ile	Gly	Thr	Leu	Asp	Gly	Ala	Asn	Val	
					690										700	
GAA	ATT	AGG	GAG	GAA	ATT	GGA	GAA	GAT	AAC	TTC	TTT	CTT	TTT	GGT	GCA	2162
Glu	Ile	Arg	Glu	Glu	Ile	Gly	Glu	Asp	Asn	Phe	Phe	Leu	Phe	Gly	Ala	
			705						710						715	
ACA	GCT	GAT	GAA	GTT	CCT	CAA	CTG	CGC	AAA	GAT	CGA	GAG	AAT	GGA	CTG	2210
Thr	Ala	Asp	Glu	Val	Pro	Gln	Leu	Arg	Lys	Asp	Arg	Glu	Asn	Gly	Leu	
			720						725						730	
TTC	AAA	CCT	GAT	CCT	CGG	TTT	GAA	GAG	GCA	AAA	CAA	TTT	ATT	AGG	TCT	2258
Phe	Lys	Pro	Asp	Pro	Arg	Phe	Glu	Glu	Ala	Lys	Gln	Phe	Ile	Arg	Ser	
			735						740						745	
GGA	GCA	TTT	GGG	ACG	TAT	GAT	TAT	AAT	CCC	CTC	CTT	GAA	TCA	CTG	GAA	2306
Gly	Ala	Phe	Gly	Thr	Tyr	Asp	Tyr	Asn	Pro	Leu	Leu	Glu	Ser	Leu	Glu	
					755										765	
GGG	AAC	TCG	GGA	TAT	GGT	CGT	GGA	GAC	TAT	TTT	CTT	GTT	GGT	CAT	GAT	2354
Gly	Asn	Ser	Gly	Tyr	Gly	Arg	Gly	Asp	Tyr	Phe	Leu	Val	Gly	His	Asp	
					770										780	
TTT	CCG	AGC	TAC	ATG	GAT	GCT	CAG	GCA	AGG	GTT	GAT	GAA	GCT	TAC	AAG	2402
Phe	Pro	Ser	Tyr	Met	Asp	Ala	Gln	Ala	Arg	Val	Asp	Glu	Ala	Tyr	Lys	
					785										795	
GAC	AGG	AAA	AGA	TGG	ATA	AAG	ATG	TCT	ATA	CTG	AGC	ACT	AGT	GGG	AGT	2450
Asp	Arg	Lys	Arg	Trp	Ile	Lys	Met	Ser	Ile	Leu	Ser	Thr	Ser	Gly	Ser	
			800						805						810	
GGC	AAA	TTT	AGT	AGT	GAC	CGT	ACA	ATT	TCT	CAA	TAT	GCA	AAA	GAG	ATC	2498
Gly	Lys	Phe	Ser	Ser	Asp	Arg	Thr	Ile	Ser	Gln	Tyr	Ala	Lys	Glu	Ile	
			815												825	
TGG	AAC	ATT	GCC	GAG	TGT	CGC	GTG	CCT	TGA	GCACACTTCT	GAACCTGGTA					2548
Trp	Asn	Ile	Ala	Glu	Cys	Arg	Val	Pro	*							
			830												835	

TCTAATAAGG ATCTAATGTT CATTGTTTAC TAGCATATGA ATAATGTAAG TTCAAGCACA 2608
 ACATGCTTTC TTATTTTCCTA CTGCTCTCAA GAAGCAGTTA TTTGTTG 2655

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 839 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Glu	Gly	Gly	Ala	Lys	Ser	Asn	Asp	Val	Ser	Ala	Ala	Pro	Ile	Ala	1	5	10	15
Gln	Pro	Leu	Ser	Glu	Asp	Pro	Thr	Asp	Ile	Ala	Ser	Asn	Ile	Lys	Tyr	20	25	30	
His	Ala	Gln	Tyr	Thr	Pro	His	Phe	Ser	Pro	Phe	Lys	Phe	Glu	Pro	Leu	35	40	45	
Gln	Ala	Tyr	Tyr	Ala	Ala	Thr	Ala	Asp	Ser	Val	Arg	Asp	Arg	Leu	Ile	50	55	60	
Lys	Gln	Trp	Asn	Asp	Thr	Tyr	Leu	His	Tyr	Asp	Lys	Val	Asn	Pro	Lys	65	70	75	80
Gln	Thr	Tyr	Tyr	Leu	Ser	Met	Glu	Tyr	Leu	Gln	Gly	Arg	Ala	Leu	Thr	85	90	95	
Asn	Ala	Val	Gly	Asn	Leu	Asp	Ile	His	Asn	Ala	Tyr	Ala	Asp	Ala	Leu	100	105	110	
Asn	Lys	Leu	Gly	Gln	Gln	Leu	Glu	Glu	Val	Val	Glu	Gln	Glu	Lys	Asp	115	120	125	
Ala	Ala	Leu	Gly	Asn	Gly	Gly	Leu	Gly	Arg	Leu	Ala	Ser	Cys	Phe	Leu	130	135	140	
Asp	Ser	Met	Ala	Thr	Leu	Asn	Leu	Pro	Ala	Trp	Gly	Tyr	Gly	Leu	Arg	145	150	155	160
Tyr	Arg	Tyr	Gly	Leu	Phe	Lys	Gln	Leu	Ile	Thr	Lys	Ala	Gly	Gln	Glu	165	170	175	
Glu	Val	Pro	Glu	Asp	Trp	Leu	Glu	Lys	Phe	Ser	Pro	Trp	Glu	Ile	Val	180	185	190	
Arg	His	Asp	Val	Val	Phe	Pro	Ile	Arg	Phe	Phe	Gly	His	Val	Glu	Val	195	200	205	

Leu Pro Ser Gly Ser Arg Lys Trp Val Gly Gly Glu Val Leu Gln Ala
 210 215 220
 Leu Ala Tyr Asp Val Pro Ile Pro Gly Tyr Arg Thr Lys Asn Thr Asn
 225 230 235 240
 Ser Leu Arg Leu Trp Glu Ala Lys Ala Ser Ser Glu Asp Phe Asn Leu
 245 250 255
 Phe Leu Phe Asn Asp Gly Gln Tyr Asp Ala Ala Ala Gln Leu His Ser
 260 265 270
 Arg Ala Gln Gln Ile Cys Ala Val Leu Tyr Pro Gly Asp Ala Thr Glu
 275 280 285
 Asn Gly Lys Leu Leu Arg Leu Lys Gln Gln Phe Phe Leu Cys Ser Ala
 290 295 300
 Ser Leu Gln Asp Ile Ile Ala Arg Phe Lys Glu Arg Glu Asp Gly Lys
 305 310 315 320
 Gly Ser His Gln Trp Ser Glu Phe Pro Lys Lys Val Ala Ile Gln Leu
 325 330 335
 Asn Asp Thr His Pro Thr Leu Thr Ile Pro Glu Leu Met Arg Leu Leu
 340 345 350
 Met Asp Asp Glu Gly Leu Gly Trp Asp Glu Ser Trp Asn Ile Thr Thr
 355 360 365
 Arg Thr Ile Ala Tyr Thr Asn His Thr Val Leu Pro Glu Ala Leu Glu
 370 375 380
 Lys Trp Ser Gln Ala Val Met Trp Lys Leu Leu Pro Arg His Met Glu
 385 390 395 400
 Ile Ile Glu Glu Ile Asp Lys Arg Phe Val Ala Thr Ile Met Ser Glu
 405 410 415
 Arg Pro Asp Leu Glu Asn Lys Met Pro Ser Met Arg Ile Leu Asp His
 420 425 430
 Asn Ala Thr Lys Pro Val Val His Met Ala Asn Leu Cys Val Val Ser
 435 440 445
 Ser His Thr Val Asn Gly Val Ala Gln Leu His Ser Asp Ile Leu Lys
 450 455 460
 Ala Glu Leu Phe Ala Asp Tyr Val Ser Val Trp Pro Thr Lys Phe Gln
 465 470 475 480
 Asn Lys Thr Asn Gly Ile Thr Pro Arg Arg Trp Ile Arg Phe Cys Ser
 485 490 495

Pro Glu Leu Ser His Ile Ile Thr Lys Trp Leu Lys Thr Asp Gln Trp
 500 505 510

Val Thr Asn Leu Glu Leu Leu Ala Asn Leu Arg Glu Phe Ala Asp Asn
 515 520 525

Ser Glu Leu His Ala Glu Trp Glu Ser Ala Lys Met Ala Asn Lys Gln
 530 535 540

Arg Leu Ala Gln Tyr Ile Leu His Val Thr Gly Val Ser Ile Asp Pro
 545 550 555 560

Asn Ser Leu Phe Asp Ile Gln Val Lys Arg Ile His Glu Tyr Lys Arg
 565 570 575

Gln Leu Leu Asn Ile Leu Gly Val Ile Tyr Arg Tyr Lys Lys Leu Lys
 580 585 590

Gly Met Ser Pro Glu Glu Arg Lys Asn Thr Thr Pro Arg Thr Val Met
 595 600 605

Ile Gly Gly Lys Ala Phe Ala Thr Tyr Thr Asn Ala Lys Arg Ile Val
 610 615 620

Lys Leu Val Thr Asp Val Gly Asp Val Val Asn Ser Asp Pro Asp Val
 625 630 635 640

Asn Asp Tyr Leu Lys Val Val Phe Val Pro Asn Tyr Asn Val Ser Val
 645 650 655

Ala Glu Met Leu Ile Pro Gly Ser Glu Leu Ser Gln His Ile Ser Thr
 660 665 670

Ala Gly Met Glu Ala Ser Gly Thr Ser Asn Met Lys Phe Ala Leu Asn
 675 680 685

Gly Cys Leu Ile Ile Gly Thr Leu Asp Gly Ala Asn Val Glu Ile Arg
 690 695 700

Glu Glu Ile Gly Glu Asp Asn Phe Phe Leu Phe Gly Ala Thr Ala Asp
 705 710 715 720

Glu Val Pro Gln Leu Arg Lys Asp Arg Glu Asn Gly Leu Phe Lys Pro
 725 730 735

Asp Pro Arg Phe Glu Glu Ala Lys Gln Phe Ile Arg Ser Gly Ala Phe
 740 745 750

Gly Thr Tyr Asp Tyr Asn Pro Leu Leu Glu Ser Leu Glu Gly Asn Ser
 755 760 765

Gly Tyr Gly Arg Gly Asp Tyr Phe Leu Val Gly His Asp Phe Pro Ser
 770 775 780

Tyr Met Asp Ala Gln Ala Arg Val Asp Glu Ala Tyr Lys Asp Arg Lys
 785 790 795 800
 Arg Trp Ile Lys Met Ser Ile Leu Ser Thr Ser Gly Ser Gly Lys Phe
 805 810 815
 Ser Ser Asp Arg Thr Ile Ser Gln Tyr Ala Lys Glu Ile Trp Asn Ile
 820 825 830
 Ala Glu Cys Arg Val Pro *
 835

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3171 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

- (A) NAME/KEY: CDS
 - (B) LOCATION: 87..3011
 - (D) OTHER INFORMATION: /product= "potato alpha-glucan"
- L-type leaf phosphorylase"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 330..3008

(ix) FEATURE:

- (A) NAME/KEY: sig_peptide
- (B) LOCATION: 87..329

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTTTTTTTTT CAACATGCAC AACAATTATT TTGATTAAAT TTTGTATCTA AAAATTTAGC 60
 ATTTTGAAAT TCAGTTCAGA GACATC ATG GCA ACT TTT GCT GTC TCT GGA TTG 113
 Met Ala Thr Phe Ala Val Ser Gly Leu
 -81 -80 -75
 AAC TCA ATT TCA AGT ATT TCT AGT TTT AAT AAC AAT TTC AGA AGC AAA 161
 Asn Ser Ile Ser Ser Ile Ser Ser Phe Asn Asn Asn Phe Arg Ser Lys
 -70 -65 -60

AAC TCA AAC ATT TTG TTG AGT AGA AGG AGG ATT TTA TTG TTC AGT TTT Asn Ser Asn Ile Leu Leu Ser Arg Arg Arg Ile Leu Leu Phe Ser Phe -55 -50 -45	209
AGA AGA AGA AGA AGA AGT TTC TCT GTT AGC AGT GTT GCT AGT GAT CAA Arg Arg Arg Arg Arg Ser Phe Ser Val Ser Ser Val Ala Ser Asp Gln -40 -35 -30 -25	257
AAG CAG AAG ACA AAG GAT TCT TCC TCT GAT GAA GGA TTT ACA TTA GAT Lys Gln Lys Thr Lys Asp Ser Ser Ser Asp Glu Gly Phe Thr Leu Asp -20 -15 -10	305
GTT TTT CAG CCG GAC TCC ACG TCT GTT TTA TCA AGT ATA AAG TAT CAC Val Phe Gln Pro Asp Ser Thr Ser Val Leu Ser Ser Ile Lys Tyr His -5 1 5	353
GCT GAG TTC ACA CCA TCA TTT TCT CCT GAG AAG TTT GAA CTT CCC AAG Ala Glu Phe Thr Pro Ser Phe Ser Pro Glu Lys Phe Glu Leu Pro Lys 10 15 20	401
GCA TAC TAT GCA ACT GCA GAG AGT GTT CGA GAT ACG CTC ATT ATA AAT Ala Tyr Tyr Ala Thr Ala Glu Ser Val Arg Asp Thr Leu Ile Ile Asn 25 30 35 40	449
TGG AAT GCC ACA TAC GAA TTC TAT GAA AAG ATG AAT GTA AAG CAG GCA Trp Asn Ala Thr Tyr Glu Phe Tyr Glu Lys Met Asn Val Lys Gln Ala 45 50 55	497
TAT TAC TTG TCT ATG GAA TTT CTT CAG GGA AGA GCT TTA CTC AAT GCT Tyr Tyr Leu Ser Met Glu Phe Leu Gln Gly Arg Ala Leu Leu Asn Ala 60 65 70	545
ATT GGT AAC TTG GGG CTA ACC GGA CCT TAT GCA GAT GCT TTA ACT AAG Ile Gly Asn Leu Gly Leu Thr Gly Pro Tyr Ala Asp Ala Leu Thr Lys 75 80 85	593
CTC GGA TAC AGT TTA GAG GAT GTA GCC AGG CAG GAA CCG GAT GCA GCT Leu Gly Tyr Ser Leu Glu Asp Val Ala Arg Gln Glu Pro Asp Ala Ala 90 95 100	641
TTA GGT AAT GGA GGT TTA GGA AGA CTT GCT TCT TGC TTT CTG GAC TCA Leu Gly Asn Gly Gly Leu Gly Arg Leu Ala Ser Cys Phe Leu Asp Ser 105 110 115 120	689
ATG GCG ACA CTA AAC TAC CCT GCA TGG GGC TAT GGA CTT AGA TAC CAA Met Ala Thr Leu Asn Tyr Pro Ala Trp Gly Tyr Gly Leu Arg Tyr Gln 125 130 135	737
TAT GGC CTT TTC AAA CAG CTT ATT ACA AAA GAT GGA CAG GAG GAA GTT Tyr Gly Leu Phe Lys Gln Leu Ile Thr Lys Asp Gly Gln Glu Glu Val 140 145 150	785
GCT GAA AAT TGG CTC GAG ATG GGA AAT CCA TGG GAA ATT GTG AGG AAT Ala Glu Asn Trp Leu Glu Met Gly Asn Pro Trp Glu Ile Val Arg Asn 155 160 165	833

GAT ATT TCG TAT CCC GTA AAA TTC TAT GGG AAG GTC ATT GAA GGA GCT Asp Ile Ser Tyr Pro Val Lys Phe Tyr Gly Lys Val Ile Glu Gly Ala 170 175 180	881
GAT GGG AGG AAG GAA TGG GCT GGC GGA GAA GAT ATA ACT GCT GTT GCC Asp Gly Arg Lys Glu Trp Ala Gly Gly Glu Asp Ile Thr Ala Val Ala 185 190 195 200	929
TAT GAT GTC CCA ATA CCA GGA TAT AAA ACA AAA ACA ACG ATT AAC CTT Tyr Asp Val Pro Ile Pro Gly Tyr Lys Thr Lys Thr Thr Ile Asn Leu 205 210 215	977
CGA TTG TGG ACA ACA AAG CTA GCT GCA GAA GCT TTT GAT TTA TAT GCT Arg Leu Trp Thr Thr Lys Leu Ala Ala Glu Ala Phe Asp Leu Tyr Ala 220 225 230	1025
TTT AAC AAT GGA GAC CAT GCC AAA GCA TAT GAG GCC CAG AAA AAG GCT Phe Asn Asn Gly Asp His Ala Lys Ala Tyr Glu Ala Gln Lys Lys Ala 235 240 245	1073
GAA AAG ATT TGC TAT GTC TTA TAT CCA GGT GAC GAA TCG CTT GAA GGA Glu Lys Ile Cys Tyr Val Leu Tyr Pro Gly Asp Glu Ser Leu Glu Gly 250 255 260	1121
AAG ACG CTT AGG TTA AAG CAG CAA TAC ACA CTA TGT TCT GCT TCT CTT Lys Thr Leu Arg Leu Lys Gln Gln Tyr Thr Leu Cys Ser Ala Ser Leu 265 270 275 280	1169
CAG GAC ATT ATT GCA CGG TTC GAG AAG AGA TCA GGG AAT GCA GTA AAC Gln Asp Ile Ile Ala Arg Phe Glu Lys Arg Ser Gly Asn Ala Val Asn 285 290 295	1217
TGG GAT CAG TTC CCC GAA AAG GTT GCA GTA CAG ATG AAT GAC ACT CAT Trp Asp Gln Phe Pro Glu Lys Val Ala Val Gln Met Asn Asp Thr His 300 305 310	1265
CCA ACA CTT TGT ATA CCA GAA CTT TTA AGG ATA TTG ATG GAT GTT AAA Pro Thr Leu Cys Ile Pro Glu Leu Leu Arg Ile Leu Met Asp Val Lys 315 320 325	1313
GGT TTG AGC TGG AAG CAG GCA TGG GAA ATT ACT CAA AGA ACG GTC GCA Gly Leu Ser Trp Lys Gln Ala Trp Glu Ile Thr Gln Arg Thr Val Ala 330 335 340	1361
TAC ACT AAC CAC ACT GTT CTA CCT GAG GCT CTT GAG AAA TGG AGC TTC Tyr Thr Asn His Thr Val Leu Pro Glu Ala Leu Glu Lys Trp Ser Phe 345 350 355 360	1409
ACA CTT CTT GGT GAA CTG CTT CCT CGG CAC GTG GAG ATC ATA GCA ATG Thr Leu Leu Gly Glu Leu Leu Pro Arg His Val Glu Ile Ile Ala Met 365 370 375	1457
ATA GAT GAG GAG CTC TTG CAT ACT ATA CTT GCT GAA TAT GGT ACT GAA Ile Asp Glu Glu Leu Leu His Thr Ile Leu Ala Glu Tyr Gly Thr Glu 380 385 390	1505

GAT CTT GAC TTG TTG CAA GAA AAG CTA AAC CAA ATG AGG ATT CTG GAT Asp Leu Asp Leu Leu Gln Glu Lys Leu Asn Gln Met Arg Ile Leu Asp 395 400 405	1553
AAT GTT GAA ATA CCA AGT TCT GTT TTG GAG TTG CTT ATA AAA GCC GAA Asn Val Glu Ile Pro Ser Ser Val Leu Glu Leu Leu Ile Lys Ala Glu 410 415 420	1601
GAA AGT GCT GCT GAT GTC GAA AAG GCA GCA GAT GAA GAA CAA GAA GAA Glu Ser Ala Ala Asp Val Glu Lys Ala Ala Asp Glu Glu Gln Glu Glu 425 430 435 440	1649
GAA GGT AAG GAT GAC AGT AAA GAT GAG GAA ACT GAG GCT GTA AAG GCA Glu Gly Lys Asp Asp Ser Lys Asp Glu Glu Thr Glu Ala Val Lys Ala 445 450 455	1697
GAA ACT ACG AAC GAA GAG GAG GAA ACT GAG GTT AAG AAG GTT GAG GTG Glu Thr Thr Asn Glu Glu Glu Glu Thr Glu Val Lys Lys Val Glu Val 460 465 470	1745
GAG GAT AGT CAA GCA AAA ATA AAA CGT ATA TTC GGG CCA CAT CCA AAT Glu Asp Ser Gln Ala Lys Ile Lys Arg Ile Phe Gly Pro His Pro Asn 475 480 485	1793
AAA CCA CAG GTG GTT CAC ATG GCA AAT CTA TGT GTA GTT AGC GGG CAT Lys Pro Gln Val Val His Met Ala Asn Leu Cys Val Val Ser Gly His 490 495 500	1841
GCA GTT AAC GGT GTT GCT GAG ATT CAT AGT GAA ATA GTT AAG GAT GAA Ala Val Asn Gly Val Ala Glu Ile His Ser Glu Ile Val Lys Asp Glu 505 510 515 520	1889
GTT TTC AAT GAA TTT TAC AAG TTA TGG CCA GAG AAA TTC CAA AAC AAG Val Phe Asn Glu Phe Tyr Lys Leu Trp Pro Glu Lys Phe Gln Asn Lys 525 530 535	1937
ACA AAT GGT GTG ACA CCA AGA AGA TGG CTA AGT TTC TGT AAT CCA GAG Thr Asn Gly Val Thr Pro Arg Arg Trp Leu Ser Phe Cys Asn Pro Glu 540 545 550	1985
TTG AGT GAA ATT ATA ACC AAG TGG ACA GGA TCT GAT GAT TGG TTA GTA Leu Ser Glu Ile Ile Thr Lys Trp Thr Gly Ser Asp Asp Trp Leu Val 555 560 565	2033
AAC ACT GAA AAA TTG GCA GAG CTT CGA AAG TTT GCT GAT AAC GAA GAA Asn Thr Glu Lys Leu Ala Glu Leu Arg Lys Phe Ala Asp Asn Glu Glu 570 575 580	2081
CTC CAG TCT GAG TGG AGG AAG GCA AAA GGA AAT AAC AAA ATG AAG ATT Leu Gln Ser Glu Trp Arg Lys Ala Lys Gly Asn Asn Lys Met Lys Ile 585 590 595 600	2129
GTC TCT CTC ATT AAA GAA AAA ACA GGA TAC GTG GTC AGT CCC GAT GCA Val Ser Leu Ile Lys Glu Lys Thr Gly Tyr Val Val Ser Pro Asp Ala 605 610 615	2177

ATG TTT GAT GTT CAG ATC AAG CGC ATC CAT GAG TAT AAA AGG CAG CTA	2225
Met Phe Asp Val Gln Ile Lys Arg Ile His Glu Tyr Lys Arg Gln Leu	
620 625 630	
TTA AAT ATA TTT GGA ATC GTT TAT CGC TAT AAG AAG ATG AAA GAA ATG	2273
Leu Asn Ile Phe Gly Ile Val Tyr Arg Tyr Lys Lys Met Lys Glu Met	
635 640 645	
AGC CCT GAA GAA CGA AAA GAA AAG TTT GTC CCT CGA GTT TGC ATA TTT	2321
Ser Pro Glu Glu Arg Lys Glu Lys Phe Val Pro Arg Val Cys Ile Phe	
650 655 660	
GGA GGA AAA GCA TTT GCT ACA TAT GTT CAG GCC AAG AGA ATT GTA AAA	2369
Gly Gly Lys Ala Phe Ala Thr Tyr Val Gln Ala Lys Arg Ile Val Lys	
665 670 675 680	
TTT ATC ACT GAT GTA GGG GAA ACA GTC AAC CAT GAT CCC GAG ATT GGT	2417
Phe Ile Thr Asp Val Gly Glu Thr Val Asn His Asp Pro Glu Ile Gly	
685 690 695	
GAT CTT TTG AAG GTT GTA TTT GTT CCT GAT TAC AAT GTC AGT GTA GCA	2465
Asp Leu Leu Lys Val Val Phe Val Pro Asp Tyr Asn Val Ser Val Ala	
700 705 710	
GAA GTG CTA ATT CCT GGT AGT GAG TTG TCC CAG CAT ATT AGT ACT GCT	2513
Glu Val Leu Ile Pro Gly Ser Glu Leu Ser Gln His Ile Ser Thr Ala	
715 720 725	
GGT ATG GAG GCT AGT GGA ACC AGC AAC ATG AAA TTT TCA ATG AAT GGC	2561
Gly Met Glu Ala Ser Gly Thr Ser Asn Met Lys Phe Ser Met Asn Gly	
730 735 740	
TGC CTC CTC ATC GGG ACA TTA GAT GGT GCC AAT GTT GAG ATA AGA GAG	2609
Cys Leu Leu Ile Gly Thr Leu Asp Gly Ala Asn Val Glu Ile Arg Glu	
745 750 755 760	
GAA GTT GGA GAG GAC AAT TTC TTT CTT TTC GGA GCT CAG GCT CAT GAA	2657
Glu Val Gly Glu Asp Asn Phe Phe Leu Phe Gly Ala Gln Ala His Glu	
765 770 775	
ATT GCT GGC CTA CGA AAG GAA AGA GCC GAG GGA AAG TTT GTC CCG GAC	2705
Ile Ala Gly Leu Arg Lys Glu Arg Ala Glu Gly Lys Phe Val Pro Asp	
780 785 790	
CCA AGA TTT GAA GAA GTA AAG GCG TTC ATT AGG ACA GGC GTC TTT GGC	2753
Pro Arg Phe Glu Glu Val Lys Ala Phe Ile Arg Thr Gly Val Phe Gly	
795 800 805	
ACC TAC AAC TAT GAA GAA CTC ATG GGA TCC TTG GAA GGA AAC GAA GGC	2801
Thr Tyr Asn Tyr Glu Glu Leu Met Gly Ser Leu Glu Gly Asn Glu Gly	
810 815 820	
TAT GGT CGT GCT GAC TAT TTT CTT GTA GGA AAG GAT TTC CCC GAT TAT	2849
Tyr Gly Arg Ala Asp Tyr Phe Leu Val Gly Lys Asp Phe Pro Asp Tyr	
825 830 835 840	

ATA GAG TGC CAA GAT AAA GTT GAT GAA GCA TAT CGA GAC CAG AAG AAA	2897
Ile Glu Cys Gln Asp Lys Val Asp Glu Ala Tyr Arg Asp Gln Lys Lys	
845 850 855	
TGG ACC AAA ATG TCG ATC TTA AAC ACA GCT GGA TCG TTC AAA TTT AGC	2945
Trp Thr Lys Met Ser Ile Leu Asn Thr Ala Gly Ser Phe Lys Phe Ser	
860 865 870	
AGT GAT CGA ACA ATT CAT CAA TAT GCA AGA GAT ATA TGG AGA ATT GAA	2993
Ser Asp Arg Thr Ile His Gln Tyr Ala Arg Asp Ile Trp Arg Ile Glu	
875 880 885	
CCT GTT GAA TTA CCT TAA AAGTTAGCCA GTTAAAGGAT GAAAGCCAAT	3041
Pro Val Glu Leu Pro *	
890	
TTTTTCCCCC TGAGGTTCTC CCATACTGTT TATTAGTACA TATATTGTCA ATTGTTGCTA	3101
CTGAAATGAT AGAAGTTTTG AATATTTACT GTCAATAAAA TACAGTTGAT TCCATTTGAA	3161
AAAAAAAAAA	3171

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 975 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Thr Phe Ala Val Ser Gly Leu Asn Ser Ile Ser Ser Ile Ser	
-81 -80 -75 -70	
Ser Phe Asn Asn Asn Phe Arg Ser Lys Asn Ser Asn Ile Leu Leu Ser	
-65 -60 -55 -50	
Arg Arg Arg Ile Leu Leu Phe Ser Phe Arg Arg Arg Arg Arg Ser Phe	
-45 -40 -35	
Ser Val Ser Ser Val Ala Ser Asp Gln Lys Gln Lys Thr Lys Asp Ser	
-30 -25 -20	
Ser Ser Asp Glu Gly Phe Thr Leu Asp Val Phe Gln Pro Asp Ser Thr	
-15 -10 -5	
Ser Val Leu Ser Ser Ile Lys Tyr His Ala Glu Phe Thr Pro Ser Phe	
1 5 10 15	
Ser Pro Glu Lys Phe Glu Leu Pro Lys Ala Tyr Tyr Ala Thr Ala Glu	
20 25 30	

Ser Val Arg Asp Thr Leu Ile Ile Asn Trp Asn Ala Thr Tyr Glu Phe
 35 40 45
 Tyr Glu Lys Met Asn Val Lys Gln Ala Tyr Tyr Leu Ser Met Glu Phe
 50 55 60
 Leu Gln Gly Arg Ala Leu Leu Asn Ala Ile Gly Asn Leu Gly Leu Thr
 65 70 75
 Gly Pro Tyr Ala Asp Ala Leu Thr Lys Leu Gly Tyr Ser Leu Glu Asp
 80 85 90 95
 Val Ala Arg Gln Glu Pro Asp Ala Ala Leu Gly Asn Gly Gly Leu Gly
 100 105 110
 Arg Leu Ala Ser Cys Phe Leu Asp Ser Met Ala Thr Leu Asn Tyr Pro
 115 120 125
 Ala Trp Gly Tyr Gly Leu Arg Tyr Gln Tyr Gly Leu Phe Lys Gln Leu
 130 135 140
 Ile Thr Lys Asp Gly Gln Glu Glu Val Ala Glu Asn Trp Leu Glu Met
 145 150 155
 Gly Asn Pro Trp Glu Ile Val Arg Asn Asp Ile Ser Tyr Pro Val Lys
 160 165 170 175
 Phe Tyr Gly Lys Val Ile Glu Gly Ala Asp Gly Arg Lys Glu Trp Ala
 180 185 190
 Gly Gly Glu Asp Ile Thr Ala Val Ala Tyr Asp Val Pro Ile Pro Gly
 195 200 205
 Tyr Lys Thr Lys Thr Thr Ile Asn Leu Arg Leu Trp Thr Thr Lys Leu
 210 215 220
 Ala Ala Glu Ala Phe Asp Leu Tyr Ala Phe Asn Asn Gly Asp His Ala
 225 230 235
 Lys Ala Tyr Glu Ala Gln Lys Lys Ala Glu Lys Ile Cys Tyr Val Leu
 240 245 250 255
 Tyr Pro Gly Asp Glu Ser Leu Glu Gly Lys Thr Leu Arg Leu Lys Gln
 260 265 270
 Gln Tyr Thr Leu Cys Ser Ala Ser Leu Gln Asp Ile Ile Ala Arg Phe
 275 280 285
 Glu Lys Arg Ser Gly Asn Ala Val Asn Trp Asp Gln Phe Pro Glu Lys
 290 295 300
 Val Ala Val Gln Met Asn Asp Thr His Pro Thr Leu Cys Ile Pro Glu
 305 310 315

Leu Leu Arg Ile Leu Met Asp Val Lys Gly Leu Ser Trp Lys Gln Ala
 320 325 330 335
 Trp Glu Ile Thr Gln Arg Thr Val Ala Tyr Thr Asn His Thr Val Leu
 340 345 350
 Pro Glu Ala Leu Glu Lys Trp Ser Phe Thr Leu Leu Gly Glu Leu Leu
 355 360 365
 Pro Arg His Val Glu Ile Ile Ala Met Ile Asp Glu Glu Leu Leu His
 370 375 380
 Thr Ile Leu Ala Glu Tyr Gly Thr Glu Asp Leu Asp Leu Leu Gln Glu
 385 390 395
 Lys Leu Asn Gln Met Arg Ile Leu Asp Asn Val Glu Ile Pro Ser Ser
 400 405 410 415
 Val Leu Glu Leu Leu Ile Lys Ala Glu Glu Ser Ala Ala Asp Val Glu
 420 425 430
 Lys Ala Ala Asp Glu Glu Gln Glu Glu Glu Gly Lys Asp Asp Ser Lys
 435 440 445
 Asp Glu Glu Thr Glu Ala Val Lys Ala Glu Thr Thr Asn Glu Glu Glu
 450 455 460
 Glu Thr Glu Val Lys Lys Val Glu Val Glu Asp Ser Gln Ala Lys Ile
 465 470 475
 Lys Arg Ile Phe Gly Pro His Pro Asn Lys Pro Gln Val Val His Met
 480 485 490 495
 Ala Asn Leu Cys Val Val Ser Gly His Ala Val Asn Gly Val Ala Glu
 500 505 510
 Ile His Ser Glu Ile Val Lys Asp Glu Val Phe Asn Glu Phe Tyr Lys
 515 520 525
 Leu Trp Pro Glu Lys Phe Gln Asn Lys Thr Asn Gly Val Thr Pro Arg
 530 535 540
 Arg Trp Leu Ser Phe Cys Asn Pro Glu Leu Ser Glu Ile Ile Thr Lys
 545 550 555
 Trp Thr Gly Ser Asp Asp Trp Leu Val Asn Thr Glu Lys Leu Ala Glu
 560 565 570 575
 Leu Arg Lys Phe Ala Asp Asn Glu Glu Leu Gln Ser Glu Trp Arg Lys
 580 585 590
 Ala Lys Gly Asn Asn Lys Met Lys Ile Val Ser Leu Ile Lys Glu Lys
 595 600 605

Thr Gly Tyr Val Val Ser Pro Asp Ala Met Phe Asp Val Gln Ile Lys
 610 615 620
 Arg Ile His Glu Tyr Lys Arg Gln Leu Leu Asn Ile Phe Gly Ile Val
 625 630 635
 Tyr Arg Tyr Lys Lys Met Lys Glu Met Ser Pro Glu Glu Arg Lys Glu
 640 645 650 655
 Lys Phe Val Pro Arg Val Cys Ile Phe Gly Gly Lys Ala Phe Ala Thr
 660 665 670
 Tyr Val Gln Ala Lys Arg Ile Val Lys Phe Ile Thr Asp Val Gly Glu
 675 680 685
 Thr Val Asn His Asp Pro Glu Ile Gly Asp Leu Leu Lys Val Val Phe
 690 695 700
 Val Pro Asp Tyr Asn Val Ser Val Ala Glu Val Leu Ile Pro Gly Ser
 705 710 715
 Glu Leu Ser Gln His Ile Ser Thr Ala Gly Met Glu Ala Ser Gly Thr
 720 725 730 735
 Ser Asn Met Lys Phe Ser Met Asn Gly Cys Leu Leu Ile Gly Thr Leu
 740 745 750
 Asp Gly Ala Asn Val Glu Ile Arg Glu Glu Val Gly Glu Asp Asn Phe
 755 760 765
 Phe Leu Phe Gly Ala Gln Ala His Glu Ile Ala Gly Leu Arg Lys Glu
 770 775 780
 Arg Ala Glu Gly Lys Phe Val Pro Asp Pro Arg Phe Glu Glu Val Lys
 785 790 795
 Ala Phe Ile Arg Thr Gly Val Phe Gly Thr Tyr Asn Tyr Glu Glu Leu
 800 805 810 815
 Met Gly Ser Leu Glu Gly Asn Glu Gly Tyr Gly Arg Ala Asp Tyr Phe
 820 825 830
 Leu Val Gly Lys Asp Phe Pro Asp Tyr Ile Glu Cys Gln Asp Lys Val
 835 840 845
 Asp Glu Ala Tyr Arg Asp Gln Lys Lys Trp Thr Lys Met Ser Ile Leu
 850 855 860
 Asn Thr Ala Gly Ser Phe Lys Phe Ser Ser Asp Arg Thr Ile His Gln
 865 870 875
 Tyr Ala Arg Asp Ile Trp Arg Ile Glu Pro Val Glu Leu Pro *
 880 885 890

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION: 1..27
 (D) OTHER INFORMATION: /function= "primer"
/label= SPL1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATTCGAAAAG CTCGAGATTT GCATAGA

27

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:
 (A) NAME/KEY: misc_feature
 (B) LOCATION: 1..27
 (D) OTHER INFORMATION: /function= "primer"
/label= SPL2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTTTATTTTC CATCGATGGA AGGTGGT

27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
 - (B) LOCATION: 1..23
 - (D) OTHER INFORMATION: /function= "primer"
- /label= SPH1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGTGCTCTC GAGCATTGAA AGC

23

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Solanum tuberosum

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1..25
- (D) OTHER INFORMATION: /function= "primer"

/label= SPH2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATAATATCCT GAATCGATGC ACTGC

25

WE CLAIM:

1 A potato plant having improved tuber cold-storage characteristics, comprising
2 a modified potato plant having a reduced level of activity of an α glucan
3 phosphorylase enzyme selected from the group consisting of α glucan L-type tuber
4 phosphorylase (GLTP) and α glucan H-type phosphorylase (GHTP) in tubers
5 produced by the plant relative to that of tubers produced by an unmodified potato
6 plant.
7

8
9 2 The potato plant of claim 1 transformed with an expression cassette having a
10 plant promoter sequence operably linked to a DNA sequence which, when transcribed
11 in the plant, inhibits expression of an endogenous α glucan phosphorylase gene
12 selected from the group consisting of a GLTP gene and a GHTP gene.
13

14 3 A potato plant having improved cold-storage characteristics, comprising a
15 potato plant transformed with an expression cassette having a plant promoter
16 sequence operably linked to a DNA sequence comprising at least 20 nucleotides of a
17 gene encoding an α glucan phosphorylase selected from the group consisting of α
18 glucan L-type tuber phosphorylase (GLTP) and α glucan H-type phosphorylase
19 (GHTP).
20

21 4 The potato plant of claim 3, wherein the encoded α glucan phosphorylase is
22 GLTP.
23

24 5 The potato plant of claim 3, wherein the encoded α glucan phosphorylase is
25 GHTP.
26

27 6 The potato plant of claim 3, wherein the DNA sequence comprises nucleotides
28 338 to 993 of SEQ ID NO: 1.
29

30 7 The potato plant of claim 3, wherein the DNA sequence comprises nucleotides
31 147 to 799 of SEQ ID NO: 3.

1
2 8 The potato plant of any one of claims 2, 3, 4, 5, 6 or 7, wherein the DNA
3 sequence is linked to the promoter sequence in an antisense orientation.
4

5 9 The potato plant of claim 4, wherein the sum of the concentration of glucose
6 and fructose in tubers of the plant measured at harvest is at least 10% lower than the
7 sum of the concentration of glucose and fructose in tubers of an untransformed plant
8 measured at harvest.
9

10 10 The potato plant of claim 4, wherein the sum of the concentration of glucose
11 and fructose in tubers of the plant measured at harvest is at least 30% lower than the
12 sum of the concentration of glucose and fructose in tubers of an untransformed plant
13 measured at harvest.
14

15 11 The potato plant of claim 4, wherein the sum of the concentration of glucose
16 and fructose in tubers of the plant measured at harvest is at least 80% lower than the
17 sum of the concentration of glucose and fructose in tubers of an untransformed plant
18 measured at harvest.
19

20 12 The potato plant of claim 4, wherein the sum of the concentration of glucose
21 and fructose in tubers of the plant stored at 4°C for about three months is at least 10%
22 lower than the sum of the concentration of glucose and fructose in tubers of an
23 untransformed plant stored under the same conditions.
24

25 13 The potato plant of claim 4, wherein the sum of the concentration of glucose
26 and fructose in tubers of the plant stored at 4°C for about three months is at least 30%
27 lower than the sum of the concentration of glucose and fructose in tubers of an
28 untransformed plant stored under the same conditions.
29

30 14 The potato plant of claim 4, wherein the sum of the concentration of glucose
31 and fructose in tubers of the plant stored at 4°C for about three months is at least 39%

1 lower than the sum of the concentration of glucose and fructose in tubers of an
2 untransformed plant stored under the same conditions.

3
4 15 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
5 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant measured
6 at harvest is at least 10% lower than the total α glucan phosphorylase activity in tubers
7 of an untransformed plant measured at harvest.

8
9 16 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
10 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant measured
11 at harvest is at least 30% lower than the total α glucan phosphorylase activity in tubers
12 of an untransformed plant measured at harvest.

13
14 17 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
15 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant measured
16 at harvest is at least 66% lower than the total α glucan phosphorylase activity in tubers
17 of an untransformed plant measured at harvest.

18
19 18 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
20 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
21 4°C for about three months is at least 10% lower than the total α glucan
22 phosphorylase activity in tubers of an untransformed plant stored under the same
23 conditions.

24
25 19 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
26 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
27 4°C for about three months is at least 30% lower than the total α glucan
28 phosphorylase activity in tubers of an untransformed plant stored under the same
29 conditions.

1 20 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
2 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
3 4°C for about three months is at least 70% lower than the total α glucan
4 phosphorylase activity in tubers of an untransformed plant stored under the same
5 conditions.

6
7 21 The potato plant of claim 5, wherein the total α glucan phosphorylase activity
8 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
9 4°C for about three months is at least 10% lower than the total α glucan
10 phosphorylase activity in tubers of an untransformed plant stored under the same
11 conditions.

12
13 22 The potato plant of claim 5, wherein the total α glucan phosphorylase activity
14 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
15 4°C for about three months is at least 28% lower than the total α glucan
16 phosphorylase activity in tubers of an untransformed plant stored under the same
17 conditions.

18
19 23 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
20 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
21 4°C for about six months is at least 10% lower than the total α glucan phosphorylase
22 activity in tubers of an untransformed plant stored under the same conditions.

23
24 24 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
25 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
26 4°C for about six months is at least 30% lower than the total α glucan phosphorylase
27 activity in tubers of an untransformed plant stored under the same conditions.

28
29 25 The potato plant of claim 4, wherein the total α glucan phosphorylase activity
30 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at

1 4°C for about six months is at least 69% lower than the total α glucan phosphorylase
2 activity in tubers of an untransformed plant stored under the same conditions.

3
4 26 The potato plant of claim 5, wherein the total α glucan phosphorylase activity
5 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
6 4°C for about six months is at least 10% lower than the total α glucan phosphorylase
7 activity in tubers of an untransformed plant stored under the same conditions.

8
9 27 The potato plant of claim 5, wherein the total α glucan phosphorylase activity
10 measured as $\mu\text{mol NADPH produced mg}^{-1} \text{ protein}^{-1} \text{ h}^{-1}$ in tubers of the plant stored at
11 4°C for about six months is at least 39% lower than the total α glucan phosphorylase
12 activity in tubers of an untransformed plant stored under the same conditions.

13
14 28 The potato plant of claim 4, wherein a chip score for tubers of the plant
15 measured at harvest is at least 5% higher than the chip scores for tubers of an
16 untransformed plant measured at harvest.

17
18 29 The potato plant of claim 4, wherein a chip score for tubers of the plant
19 measured at harvest is at least 30% higher than the chip scores for tubers of an
20 untransformed plant measured at harvest.

21
22 30 The potato plant of claim 4, wherein a chip score for tubers of the plant
23 measured at harvest is at least 46% higher than the chip scores for tubers of an
24 untransformed plant measured at harvest.

25
26 31 The potato plant of claim 5, wherein a chip score for tubers of the plant
27 measured at harvest is at least 5% higher than the chip scores for tubers of an
28 untransformed plant measured at harvest.

1 32 The potato plant of claim 5, wherein a chip score for tubers of the plant
2 measured at harvest is at least 10% higher than the chip scores for tubers of an
3 untransformed plant measured at harvest.

4
5 33 The potato plant of claim 4, wherein a chip score for tubers of the plant stored
6 at 4°C for about three months is at least 5% higher than the chip scores for tubers of
7 an untransformed plant stored under the same conditions.

8
9 34 The potato plant of claim 4, wherein a chip score for tubers of the plant stored
10 at 4°C for about three months is at least 30% higher than the chip scores for tubers of
11 an untransformed plant stored under the same conditions.

12
13 35 The potato plant of claim 4, wherein a chip score for tubers of the plant stored
14 at 4°C for about three months is at least 89% higher than the chip scores for tubers of
15 an untransformed plant stored under the same conditions.

16
17 36 The potato plant of claim 5, wherein a chip score for tubers of the plant stored
18 at 4°C for about three months is at least 5% higher than the chip scores for tubers of
19 an untransformed plant stored under the same conditions.

20
21 37 The potato plant of claim 5, wherein a chip score for tubers of the plant stored
22 at 4°C for about three months is at least 10% higher than the chip scores for tubers of
23 an untransformed plant stored under the same conditions.

24
25 38 The potato plant of claim 4, wherein a chip score for tubers of the plant stored
26 at 4°C for about four months is at least 5% higher than the chip scores for tubers of an
27 untransformed plant stored under the same conditions.

28
29 39 The potato plant of claim 4, wherein a chip score for tubers of the plant stored
30 at 4°C for about four months is at least 30% higher than the chip scores for tubers of
31 an untransformed plant stored under the same conditions.

1
2 40 The potato plant of claim 4, wherein a chip score for tubers of the plant stored
3 at 4°C for about four months is at least 89% higher than the chip scores for tubers of
4 an untransformed plant stored under the same conditions.

5
6 41 The potato plant of claim 5, wherein a chip score for tubers of the plant stored
7 at 4°C for about four months is at least 5% higher than the chip scores for tubers of an
8 untransformed plant stored under the same conditions.

9
10 42 The potato plant of claim 5, wherein a chip score for tubers of the plant stored
11 at 4°C for about four months is at least 25% higher than the chip scores for tubers of
12 an untransformed plant stored under the same conditions.

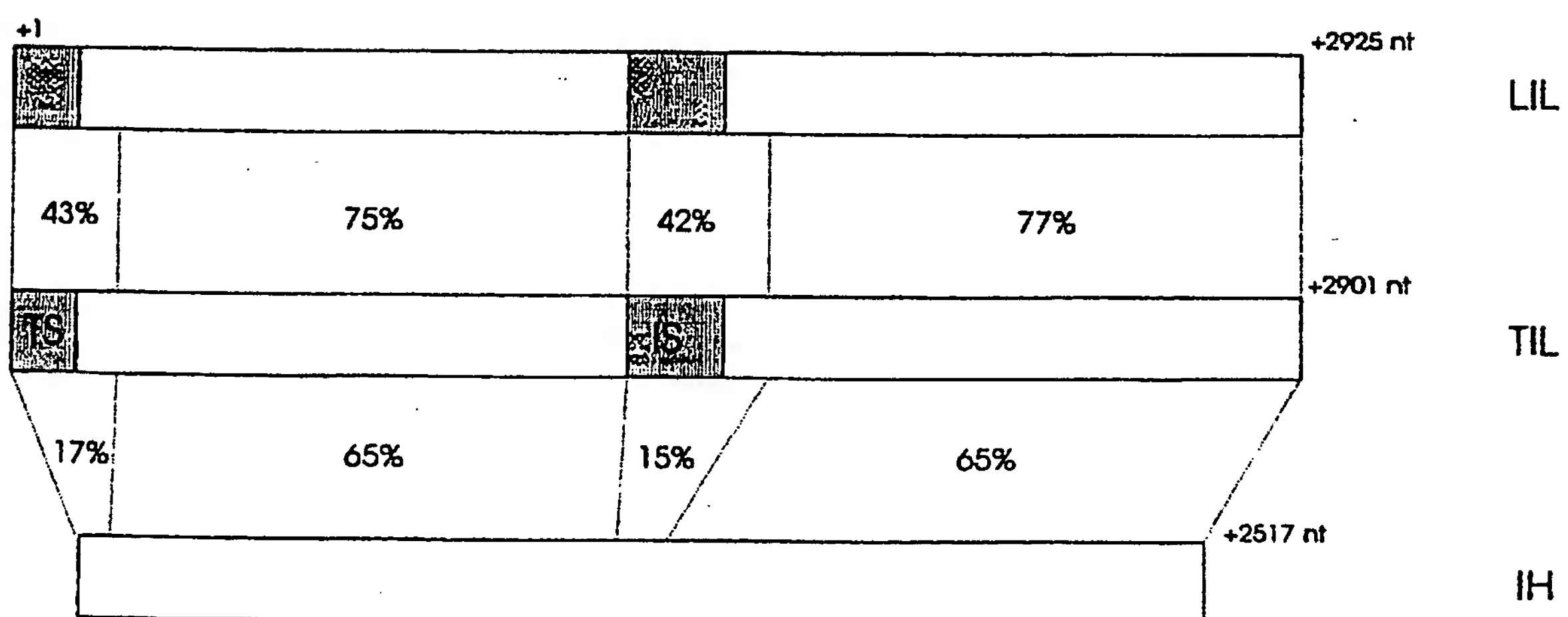
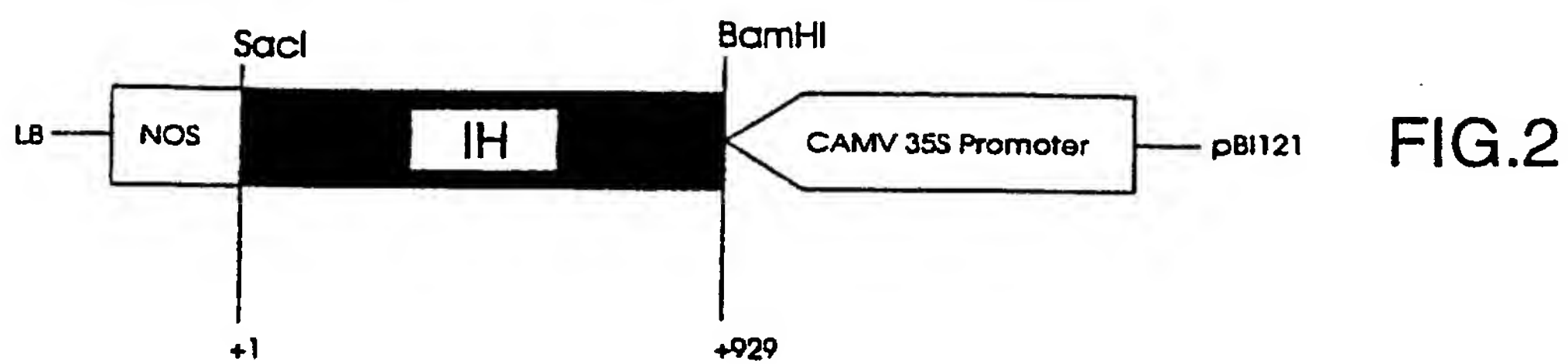
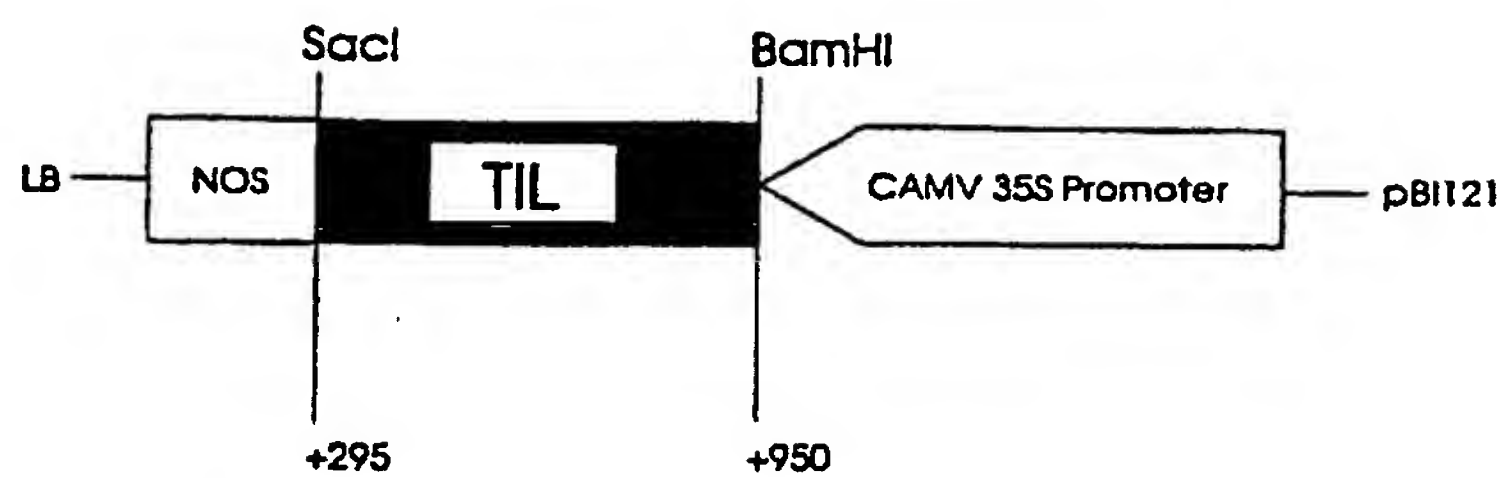
13
14 43 A method for improving the cold-storage characteristics of a potato tuber,
15 comprising providing a potato plant which has been modified to reduce the level of
16 activity in the tubers of an α glucan phosphorylase enzyme selected from the group
17 consisting of α glucan L-type tuber phosphorylase (GLTP) and α glucan H-type
18 phosphorylase (GHTP).

19
20 44 The method of claim 43, comprising:
21 introducing into the potato plant an expression cassette having a plant promoter
22 sequence operably linked to a DNA sequence which, when transcribed in the plant,
23 inhibits expression of an endogenous α glucan phosphorylase gene selected from the
24 group consisting of a GLTP gene and a GHTP gene.

25
26 45 A method for improving the cold-storage characteristics of a potato tuber,
27 comprising:
28 introducing into a potato plant an expression cassette having a plant promoter
29 sequence operably linked to a DNA sequence comprising at least 20 nucleotides of a
30 gene encoding an α glucan phosphorylase selected from the group consisting of GLTP
31 and GHTP.

- 1 46 The method of claim 45, wherein the encoded α glucan phosphorylase is
2 GLTP.
3
- 4 47 The method of claim 45, wherein the encoded α glucan phosphorylase is
5 GHTP.
6
- 7 48 The method of claim 45, wherein the DNA sequence comprises nucleotides
8 338 to 993 of SEQ ID. NO: 1.
9
- 10 49 The method of claim 45, wherein the DNA sequence comprises nucleotides
11 147 to 799 of SEQ ID. NO: 3.
12
- 13 50 The method of any one of claims 44, 45, 46, 47, 48 or 49 wherein the DNA
14 sequence is linked to the promoter sequence in an antisense orientation.
15
16

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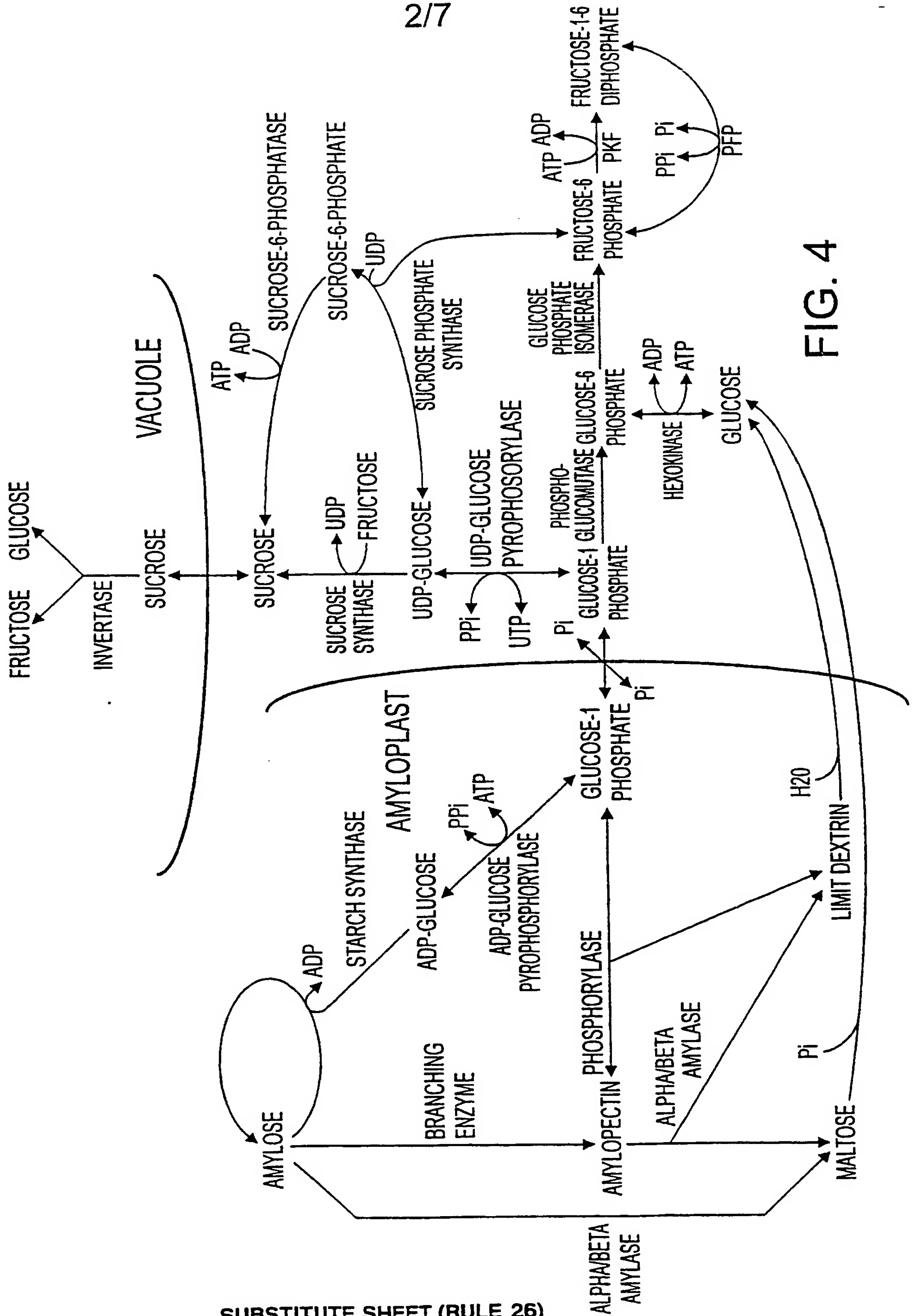


FIG. 4

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ELPKAYYA-TAESVRDTLI INWNATYEFYEKMNVKQAYYLSMEFLQGRAL	49
ELPKAFFA-TAQSVRDSLL INWNATYDIYEKLNMKQAYYLSMEFLQGRAL	49
EPLQAYYAATADSVRDRLIKQWNDTYLHYDKVNPQTYYLSMEYLQGRAL	50
* . * . . * * * . * * * * * * . * . * * * * * * . * * * * * * . * * * * * *	
LNAIGNLGLTGPYADALTKLGYSLEDVARQEPDAALGNGGLGRLASCFLD	99
LNAIGNLELTGDFAEALKNLGHNLENVASQEPDAALGNGGLGRLASCFLD	99
TNAVGNLDIHNAYADALNKLGOQLEEVVEQEKDAALGNGGLGRLASCFLD	100
* * . * * * * . * * . . * * * * . *	
SMATLNYPAWGYGLRYQYGLFKQLITKDGQEEVAENWLEMGNPWEIVRND	149
SLATLNYPAWGYGLRYKYGLFKQRITKDGQEEVAEDWLEIGSPWEVVRND	149
SMATLNLPAWGYGLRYRYGLFKQLITKAGQEEVPEDWLEKFSWEIVRHD	150
* . * . * . * * * * * * . * * * . * * * . * *	
ISYPVKFYGKVIEGADGRKEWAGGEDITAVAYDVPIPGYKTKTTINLRLW	199
VSYPPIKFYGKVSTGSDGKRYWIGGEDIKAVAYDVPIPGYKTRTTISLRLW	199
VVFPIRFFGHVEVLPSGSRKWVGGEVLQALAYDVPIPGYRTKNTNSLRLW	200
. . * . . * . * . * . . * . . * * * * . * . * * * * * * * * . * . . * . * * * * *	
TTKLAAEAFDLYAFNNGDH	218
STQVPSADFDLSAFNAGEH	218
EAKASSEDfNLFLFNDGQY	219
. * . * * * . * . .	

FIG.5

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GAAC TTCCCAAGGCATACTATGCA---ACTGCAGAGAGTGTTTCGAGATAC 47
GAGCTCCCTAAGGCATTCTTTGCA---ACAGCTCAAAGTGTTTCGTGATTC 47
GAGCCACTACAAGCATACTATGCTGCTACTGCTGACAGTGTTTCGTGAT-C 49
.* * .***.***.***. **.*. * *****.*** *

GCTCATTATAA-ATTGGAATGCCACATACGAATTCTATGAAAAGATGAAT 96
GCTCCTTATTA-ATTGGAATGCTACGTATGATATTTATGAAAAGCTGAAC 96
GCTTGATCAAACAATGGAATGACACCTATCTTCATTATGACAAAGTTAAT 99
*** . * . * .***** ** ** . . ***** ** . * .**

GTAAAGCAGGCATATTACTTGTCTATGGAATTTCTTCAGGGAAGAGCTTT 146
ATGAAGCAAGCGTACTATCTATCCATGGAATTTCTGCAGGGTAGAGCATT 146
CCAAAGCAAACATACTACTTATCAATGGAGTATCTCCAGGGGCGAGCTTT 149
.*****.*** ** .** *****.*** *****. *****.***

ACTCAATGCTATTGGTAACTTGGGGCTAAC-CGGACCTTATGCAGATGCT 195
GTTAAATGCAATTGGTAACTCTGGAGCTTAC-TGGTGACTTTGCGGAAGCT 195
GACAAATGCAGTTGGAACTTAGA-CATCCACAATGCATATGCTGATGCT 198
. *****.*****.*** * . * . * *****.***.***

TTAACTAAGCTCGGATACAGTTTAGAGGATGTAGCCAGGCAGGAACCGGA 245
TTGAAAACCTTGGCCACAATCTAGAAAATGTGGCTTCTCAGGAACCAGA 245
TTAAACAACTGGGTCAGCAGCTTGAGGAGGTCGTTGAGCAGGAAAAAGA 248
.* **.* **.* * . . * ..*.*** * . .*****.***

TGCAGCTTTAGGTAATGGAGGTTTAGGAAGACTTGCTTCTTGCTTTCTGG 295
TGCTGCTCTTGGAATGGGGGTTTGGGACGGCTTGCTTCCTGTTTCTTG 295
TGCAGCATTAGGAATGGTGGTTTAGGAAGGCTCGCTTCATGCTTTCTTG 298
.. * .**.******.*****.*** * .** ***** ** ** * .*

ACTCAATGGCGACACTAACTACCCTGCATGGGGCTATGGACTTAGATAC 345
ACTCTTTGGCAACACTAACTACCCAGCATGGGGCTATGGACTTAGGTAC 345
ATTCCATGGCCACATTGAACCTTCCAGCATGGGGTTATGGCTTGAGGTAC 348
* ** .***** *** * .*** . **.****** ***** * .**.****

CAATATGGCCTTTTCAAACAGCTTATTACAAAAGATGGACAGGAGGAAGT 395
AAGTATGGTTTATTTAAGCAACGGATTACAAAAGATGGTCAGGAGGAGGT 395
AGATATGGACTTTTAAAGCAGCTTATCACAAGGCTGGGCAAGAAGAAGT 398
.***** * .** **.***.*.*** **.******. * *****.***.***.***

TGCTGAAAATTGGCTCGAGATGGGAAATCCATGGGAAATTGTGAGGAATG 445
GGCTGAAGATTGGCTTGAAATTGGCAGTCCATGGGAAGTTGTGAGGAATG 445
TCCTGAAGATTGGTTGGAGAAATTTAGTCCCTGGGAAATTGTAAGGCATG 448
. *****.***** * **.*..... * .*** *****.*****.*** **

ATATTTTCGTATCCCGTAAAATTCTATGGGAAGGTCATTGAAGGAGCTGAT 495
ATGTTTCATATCCTATCAAATTCTATGGAAAAGTCTCTACAGGATCAGAT 495
ATGTTGTCTTTCCTATCAGGTTTTTTGGTCATGTTGAAGTCCTCCCTTCT 498
.*. * .* . * * .** * .***. * .** . . . * . . *

FIG. 6A

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GGGAGGAAGGAATGGGCTGGCGGAGAAGATATAACTGCTGTTGCCTATGA	545
GGAAAGAGGTATTGGATTGGTGGAGAGGATATAAAGGCAGTTGCGTATGA	545
GGCTCGCGAAAATGGGTTGGTGGAGAGGTCCTACAGGCTCTTGCAATATGA	548
** . * ...*.*** . *** *****.*. ** .** . ***** *****	
TGTCCCAATACCAGGATATAAAACAAAAACAACGATTAACCTTCGATTGT	595
TGTTCCCATACCAGGGTATAAGACCAGAACCACAATCAGCCTTCGACTGT	595
TGTGCCAATTCCAGGATACAGAACTAAAAACACTAATAGTCTTCGTCTCT	598
*** ** *.*****.* *..* *.* **.*. *. *****. * *	
GGACAAC-AAAGCTAGCTGCAGAAGCTTTTGATTTATATGCTTTTAACAA	644
GGTCTAC-ACAGGTTCCATCAGCGGATTTTGATTTATCTGCTTTCAATGC	644
GGGAAGCCAAAGCAAGCT-CTGAGGATTTCAACTTGTTTCTGTTTAATGA	647
** . ..* * ** .. *. *.* . * *** . * **.* * * .** ** .	
TGGAGACCATGC	656
TGGAGAGCACAC	656
TGGACAGTATGA	659
***** * * .	

FIG.6B

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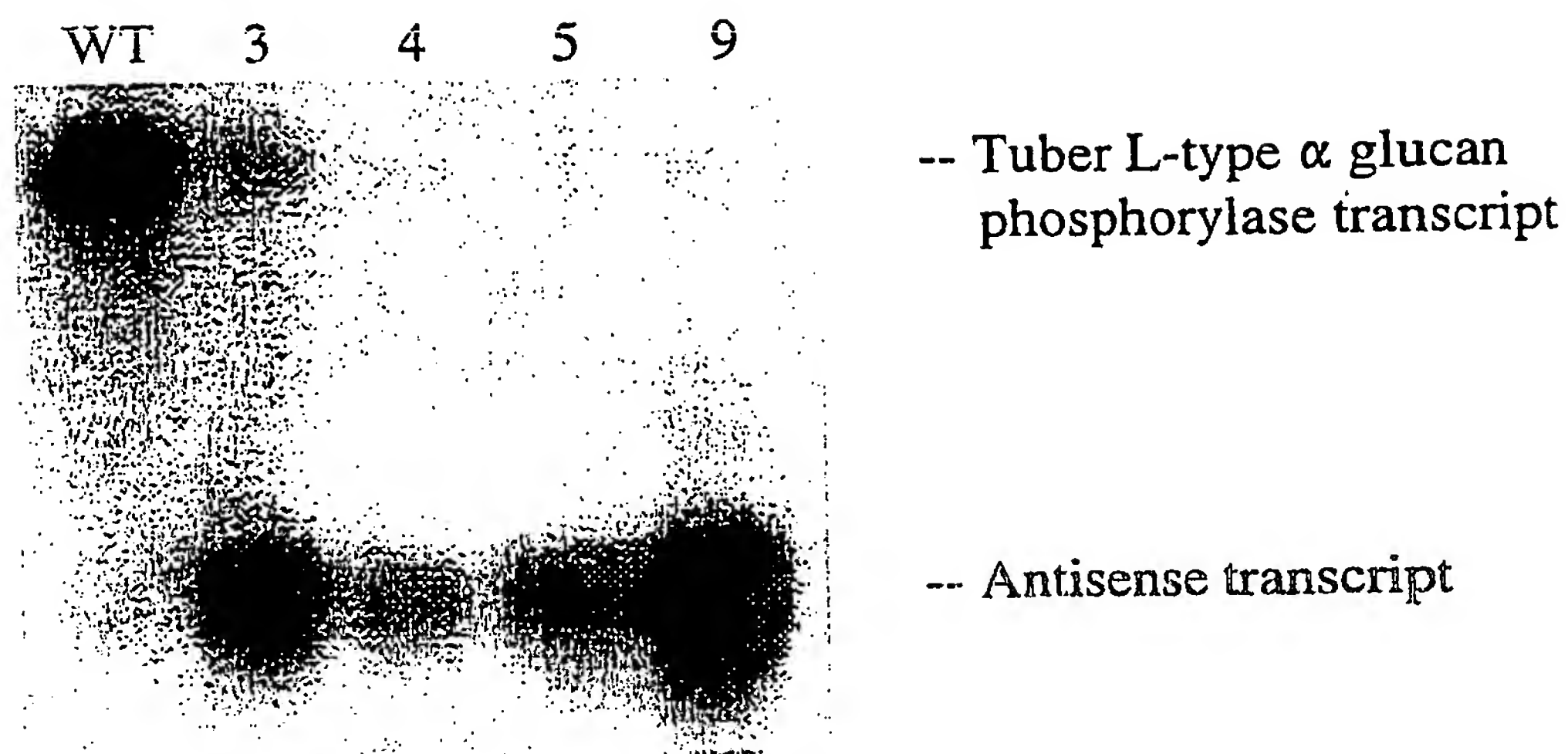


FIG.7

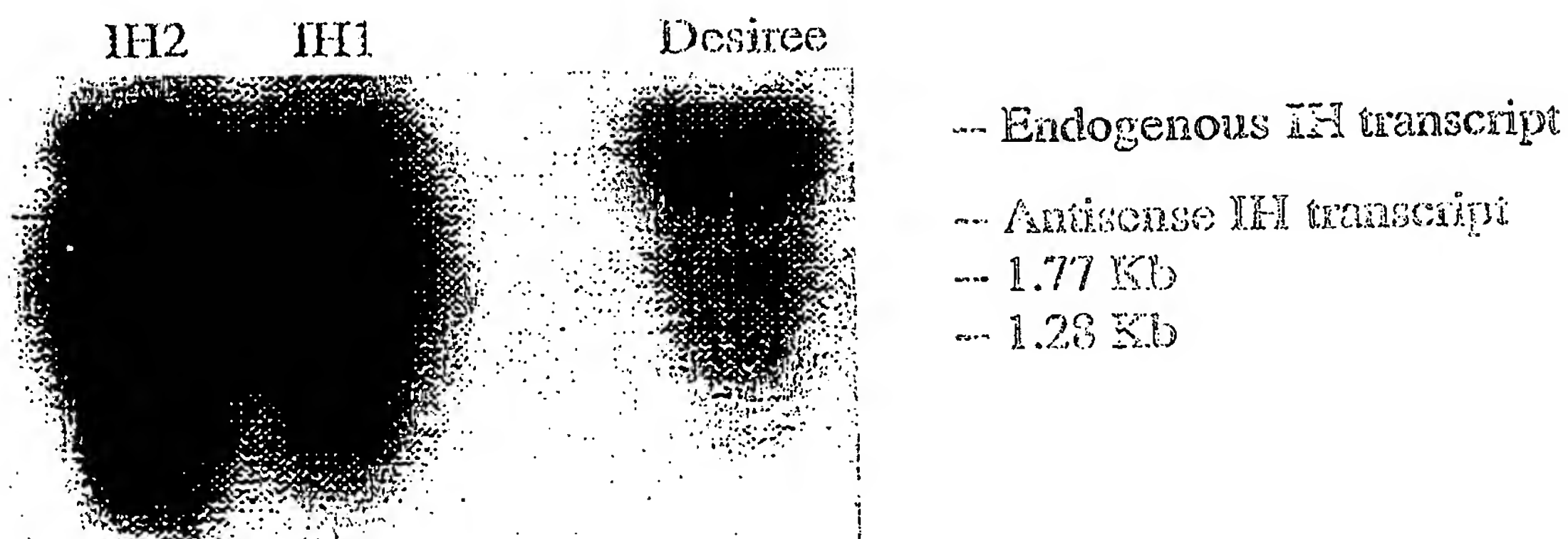


FIG.8

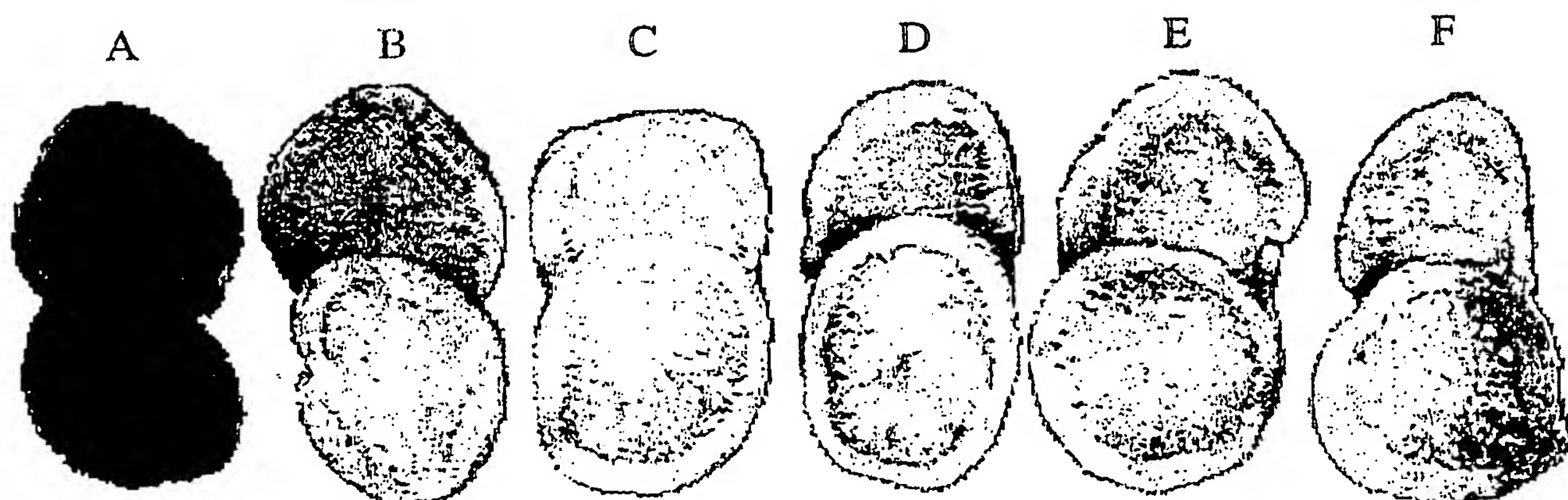


FIG.9

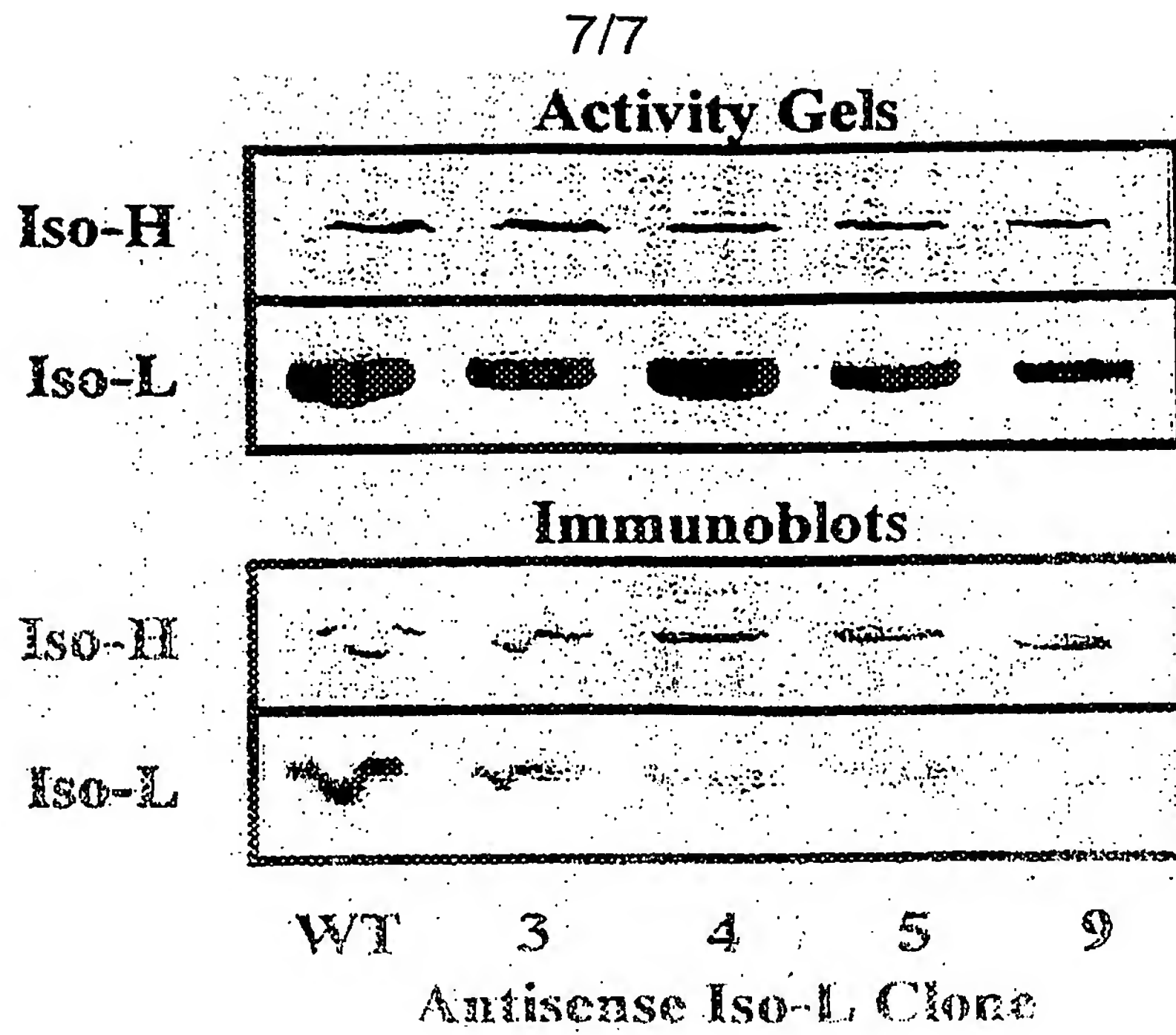


FIG.10

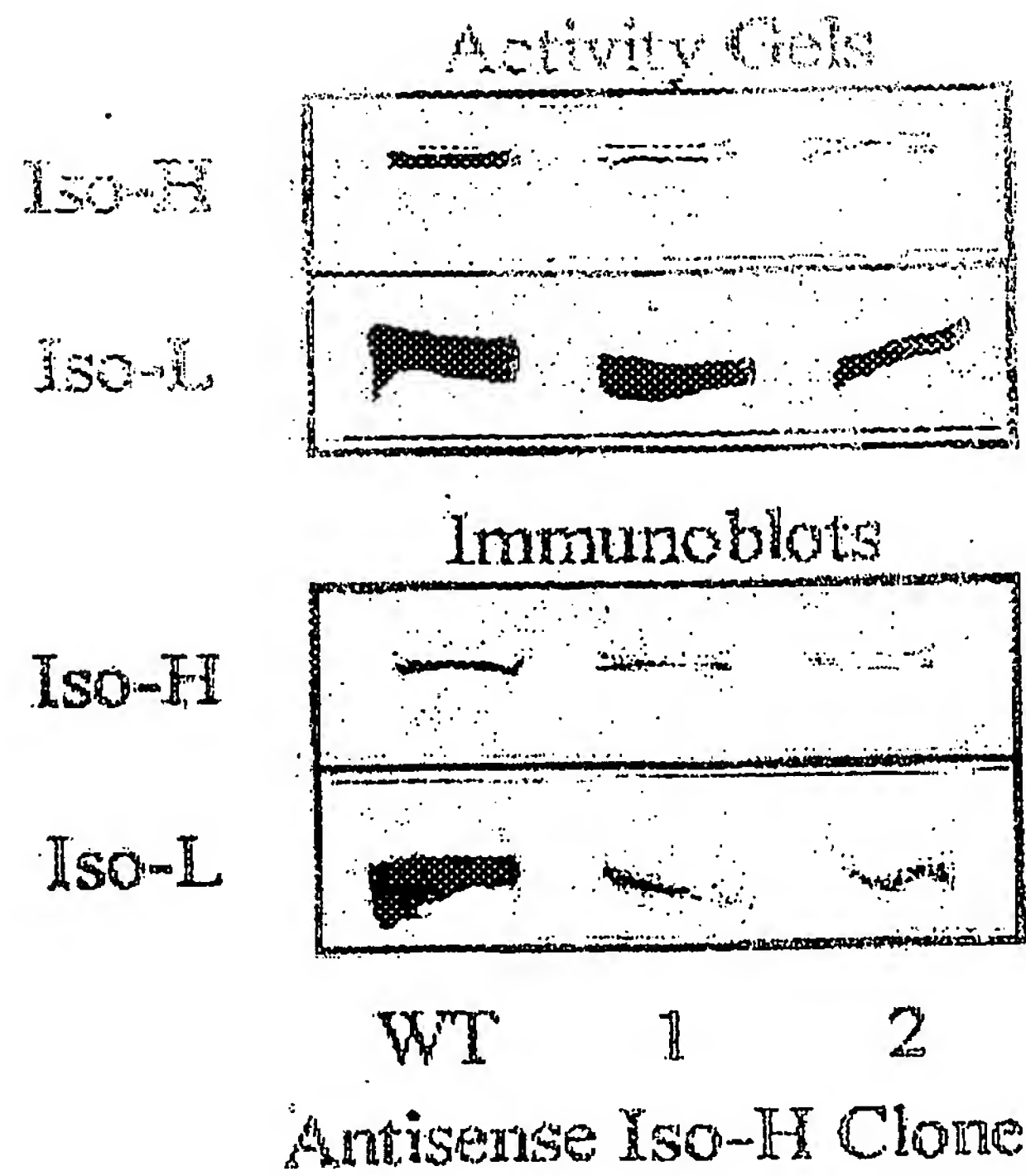


FIG.11

INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/CA 98/00055

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 A01H5/00 //C12N9/10

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP 0 812 917 A (JAPAN TOBACCO INC) 17 December 1997 see page 5, line 18 - line 28 & WO 97 24449 A ---	1-50
A	WO 90 12876 A (DANSKE SPRITFABRIKKER) 1 November 1990 see page 1, paragraph 4 ---	
A	WO 94 28149 A (MONSANTO CO ;BARRY GERARD FRANCIS (US); KISHORE GANESH MURTHY (US)) 8 December 1994 cited in the application ---	
A	WO 94 00563 A (INST GENBIOLOGISCHE FORSCHUNG ;SONNEWALD UWE (DE)) 6 January 1994 see the whole document -----	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

15 May 1998

Date of mailing of the international search report

04/06/1998

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Chakravarty, A